Bacterial adherence to separated modular components in joint prosthesis. A clinical study.

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ABSTRACT (250 words)

Background: Bacterial adherence on total joint replacement implants may lead to biofilm formation and implant-related osteoarticular infection. It is unclear if different biomaterials in the prosthetic components are more prone to facilitate this bacterial adherence, although polyethylene component exchange in the modular systems has been clinically utilized in the early management of these infections. To clarify if the amount of clinically adhered microorganisms was related to the material or the component, we investigated retrieved implants from infected joint replacements.

Material and methods: 32 patients were revised after confirmed implant-related infection through positive cultures. A number of 87 total joint components (hip and knee) were obtained and separately sonicated after surgical retrieval, following a previously published protocol. Cultures were quantified, and detected CFUs were adjusted according to the component surface, and compared based on the component material and location.

Results: Variable adherence of bacteria to chrome cobalt alloys, UHMWPE, hydroxyapatite coated components and titanium alloys. The commonest isolated organisms were Staphylococcus epidermidis (23 out of 87 components) and Staphylococcus aureus (10/87). Twelve components did not show any microorganism adhered despite location in an infected joint, with positive cultures in other components. A mixed linear model adjusted for random effects (the random effect being the infected patient) obtained convergence for the CFU/mm² variable but could not confirm a significantly higher adherence to a particular component or to a particular biomaterial. Therefore, the bacterial adherence primarily depends on the infective microorganism and the response of each individual patient, rather than materials or components.
INTRODUCTION

Implant related infection is a dreadful menace to the survivorship and successful outcome of total joint replacement. Although the overall rate of implant infection is under 2-3% in most reports and registries [1], the total number of infected implants is high, and the clinical and economic consequences of significant importance [2]. Increasing interest of the Orthopaedic community is being placed on implant related infection in view of the yearly growing rates of total joint replacement both USA and European countries [3, 4] and future predictions [5, 6].

Bacterial adherence and subsequent biofilm formation on total joint replacement implants are at the origin and maintenance of implant-related osteoarticular infection [7, 8], and are currently considered the key phenomenon in the pathogenesis of these infections, with further implications in the diagnosis and management of the patients [6, 9, 10].

Efforts to diagnose the causative agent on colonized implants have recently been renewed with the introduction of retrieved implant sonication protocols in some laboratories [9, 11, 12]. With this technique, the presence of microorganisms has been diagnosed with higher sensitivity and specificity [12] in certain settings, and even a quantitative approach has been suggested as a new criterion for infection diagnosis [9].

Nevertheless, the component with the predominant bacterial adherence in the prosthetic joint is currently unknown, even if it may be the origin and the cause of initiating and maintaining the joint infection. Different biomaterials in the components are claimed to differently suffer from microorganism adherence and biofilm formation, even justifying clinical decisions such as component exchange in some cases of acute prosthetic joint infection. However, only experimental early studies have considered the preferred adherence of microorganisms to polymethylmetacrilate (PMMA) [13] compared with polyethylene, stainless steel and chrome-cobalt alloys. Chrome-cobalt alloys also showed experimentally a significantly higher infection susceptibility than titanium alloys [14], and rough titanium alloys were more prone to infection
than polished [15]. Yet clinical studies confirming or refuting a preferred bacterial adherence to a certain material or component are lacking.

Although significant efforts are being placed by the material scientists to increase the biomaterial resistance to bacterial adherence [16], clinical data about the bacterial colonization of the different biomaterials that constitute the implanted prosthetic components are not available. This knowledge would certainly help in directing biomaterial scientists and implant designers towards the development of infection-resistant orthopaedic implants.

In this study, we aimed to isolate and quantify the adherent microorganisms to each individual component retrieved from infected total hip and knee replacements, so as to analyze the different bacterial adherence to each one of the retrieved parts, and, in consequence, the different bacterial adherence to each biomaterial.

MATERIAL AND METHODS

Patients and samples

A total of 87 total joint components (51 hip and 36 knee components) from 32 patients (20 hip and 12 knee arthroplasties) with clinical and microbiological diagnosis of implant-related infection were included in the study. Components under study included 6 femoral heads, 18 femoral stems, 14 metal cup shells, 13 acetabular liners, 9 femoral knee components, 4 all-polyethylene patellas, 11 tibial trays, and 12 tibial polyethylene components. Material in the component surface was chrome-cobalt (CC) alloys in 33, ultra-high molecular weight polyethylene (UHMWPE) in 27, hydroxyapatite (HA) in 17 (5 of which were fully coated), and titanium (Ti) alloys in 10.

Sample processing
Patients were revised with a diagnosis of infected total joint (hip or knee) replacement at Hospital Fundación Jiménez Díaz or at Hospital La Princesa, both being University Hospitals in Madrid (Spain). All patients gave informed consent to the surgery and to the study of any obtained material from the joint. No antibiotic was used preoperatively, and any antibiotic given before admission were stopped at least 24h before surgery. Implants retrieved at revision surgery were gently irrigated to rinse rests of blood in the surgical table under sterile conditions, separated into parts, and individually placed in sterile bags (the part inside one bag, and this inside a second one that was handled to the circulating nurse). The material was then submitted to the Microbiology laboratory for processing. In case processing was not immediately available, the specimens were kept overnight at 4ºC in a freezer located at the surgical area during less than 24 hours. At the reference laboratory (FJD), samples were aseptically removed from the original bags and located into new sterile plastic bags using sterile material throughout the process. Fifty ml of buffer phosphate were added per component and bag, and bags were then closed. Samples were then sonicated according to a previously described protocol [12]. To avoid contamination problems, water in the sonicator was discharged and replaced after each sonication, and bags were also carefully investigated for any leakage before and after sonication, and the component discarded in case of any damage in the sterile bag. Samples were inoculated quantitatively (10 µl/plate) onto Tryptic-soy 5 % sheep blood agar (TS), Chocolate agar (CH), Schaedler 5 % sheep blood agar (SCH), McConkey agar (MC), Middlebrook 7H10 agar (MIDD) and Sabouraud-Chloramphenicol agar (SC), all from bioMérieux (Marcy L’Etoile, France). TS, CH, SCH and MC were incubated at 37ºC in 5 % CO2 atmosphere during 7 days, MIDD was incubated under the same conditions during 3 weeks, and SC was incubated at 30ºC in normal atmosphere during 4 weeks, as previously reported [12]. Cultures were quantified by counting the number of colonies that grew in the plate, adjusted to number of Colony Forming Units (CFU)/ml, and then adjusted to
CFU/sample. Isolated organisms were identified according to commonly reported criteria using biochemical tests (coagulase, oxidase) and commercial identification galleries (API System galleries, bioMérieux, Marcy L’Etoile, France). We defined mixed anaerobic microbiota as the presence of more than 2 different species of anaerobic bacteria. Antimicrobial susceptibility testing was performed using a disc-plate assay according to CLSI standards.

**Surface quantification**

The individual components of 6 retrieved joint implants (3 hip replacements and 3 knee replacements) were separately scanned using a Picza 3D Laser Scanner LPX-60 (Roland DG Corporation, Japan). All the components were scanned in the plane mode at 0.6 mm pitch, except for femoral heads, which were scanned in the rotary mode. 3D point cloud data were converted into polygon meshes using Dr. PICZA3 software for further file conversion and analysis. Measurements of the scanned surfaces (in mm²) were obtained for each individual component working with PixformTM Pro software (Figures 4 and 5), and the average of measured components included different designs and sizes. Roughness or surface porosity varying among implants could slightly influence the surface in a micrometric scale, not the average measurements used in the study.

**Statistical analysis**

Considering the event of bacterial adherence an independent effect, descriptive and comparative (Kruskal-Wallis, Mann-Whitney, and Chi square tests) statistics were used (CFU/mm² variables did not follow a normal distribution in the Kolmogorov-Smirnov test) in a SPSS 17.0 software (SPSS, Chicago IL, USA).

However, in view that the event of bacterial adherence was not independent from the patient with the infection (different components are at risk of adherence in a single patient), mixed
linear models with random effects were prepared with SAS software 9.3 (SAS Institute Inc., Cary NC, USA), the infected patient being considered the random effect. For both TKA and THA components and for each of them, the models are adjusted for the number of CFU/mm² and the component and the material were considered fixed effects. For samples with >100,000 CFU/ml we have used 100,000 CFU/sample for the different calculations. For samples with <500 CFU/sample we used 0 CFU/sample.

RESULTS

Components with positive cultures

Culture was positive in 65 of the 87 components, of which 12 showed more than one microorganism. The average delay before processing was 7.6 ± 7.3 hours. The overall CFU/mm² averaged 2.45 with a SD of 4.05. This occurred in 32 patients with one infected joint (single hip or knee). The etiology of these 32 infections was Gram-positive cocci in 23 cases, aerobic Gram-negative bacilli in 11 cases, anaerobes in 3 cases, Mycobacterium sp. in 2 cases and fungi in one case. Microbiological and component information is displayed in Table 1. S. epidermidis (present on 23 components from 9 patients) and S. aureus (present on 11 components from 5 patients) were the most frequently isolated microorganisms. Polymicrobial isolates were found with S. epidermidis, S. aureus, P. aeruginosa and S. lugdunensis.

Components with negative cultures

Culture of sonicate was negative in 18 components retrieved from 12 patients (5 infected hips and 7 infected knees). Among these 18 components without isolated bacteria, 9 of them were UHMWPE components (out of 27 UHMWPE components in the whole series), 7 manufactured with chrome cobalt alloys (out of 33 chrome cobalt components), 2 HA coated (from 17 HA coated components), and none of titanium alloys (all 10 titanium alloy components in the series were found with adherent microorganisms). Maximum bacterial counts in the 5 infected hips
with at least one component without bacteria were found in stems and cups of chrome-cobalt, and titanium alloys with and without HA coating. The highest bacterial counts in the 7 infected knees with at least one component without bacteria were found in chrome-cobalt tibial trays. Table 2 displays a microbiological descriptive analysis of infected joints that presented one or two components without microorganisms after sonication and culture.

*Influence of prostheses type, component and biomaterial*

The distribution of isolated bacteria per surface unit of implant (CFU/mm²) is shown in Figure 1, separating hips and knees in our series. The distribution of individual components is shown in Figure 2; significant differences were found in the adhered CFU/mm² among different components (p=0.018, Kruskal-Wallis). The distribution separated for the different biomaterials is shown in Figure 3; the presence of positive cultures was also different among different biomaterials when independently considered (p=0.005, Kruskal-Wallis).

Figures 1, 2, and 3 display the great variability found in bacterial adherence. However, taking into account that these were not independent events, but rather related to specific microorganisms infecting individual patients, the association of different adherence per surface (CFU/mm²) to components and materials was studied individually within each infected joint and patient through a mixed linear model, where the random effect was the patient sustaining the infection. Models for all components, for hip components alone and for knee components alone were run, complying with the convergence criterion after 3 iterations. After statistical analysis by means of this linear model, both component type and biomaterial type failed to prove a significant difference in the conjoined and the one-to-one comparison. These final results confirmed that fixed effects such as component type or biomaterial in the component did not differ when a powerful random effect generated by an individual infected patient with a specific microorganism was considered.
DISCUSSION

The development of implant sonication as a diagnostic tool for prosthetic joint infection may be an important advance in the management of the patients, increasing the sensitivity of conventional techniques. The knowledge of the etiology of the infection is of great importance because it allows selecting the best possible antimicrobial therapy [9, 10]. Sonication even allows a quantitative assay that, theoretically, could lead to establish quantitative criteria for a definitive evaluation of the microbiological results [6, 9, 11]. Nevertheless, some data suggest that clinical infection could be diagnosed with low bacterial counts [12], and even in some cases no organisms can be detected using techniques based on conventional cultures, so other approaches, like detection of organisms using molecular biology techniques, will be necessary [17]. Many factors could influence these results, but delay in processing (if samples were properly refrigerated) had not shown a statistically significant effect on the recovery of organisms [12].

Results of our sonication of disassembled components were statistically analyzed by means of a mixed linear model and highlight the dominant influence of each joint infection in each individual patient when analyzing bacterial counts.

In spite of this conclusion about the prevailing effect of the individual patient and microorganism, hip infections included in our series were apparently more severe cases with more isolated microorganisms per surface unit when compared to knees (Figure 1). Moreover, hip infections produced almost no bacterial adherence to femoral head components (Figure 2), while knee infections basically seeded on the tibial trays in our series (Figure 2). Additionally, bacterial adherence on each type of biomaterial (Figure 3) also displayed a trend towards less adherence to UHMWPE in the knee and to HA in the hip retrieved implants, as suggested by independent comparisons. As a study limitation, we observed the high variability of microorganisms and patients that did not permit in our series to conclude any further. Another
limitation could be the lack of knowledge about the sonication effect in the different materials found in clinical samples. However, sonication has been largely used with in vitro and in vivo models of bacterial adherence and biofilm [18-21], without proving differences among materials. We performed fluorescent stains after sonication (as a test of sonication efficacy in titanium alloy and polyethylene, data not published) and no remaining bacteria were detected. Our results hold a relevant research consequence, as many new proposals of materials with a supposed resistance to infection based on blocking the adherence of certain microorganisms to a modified material may be unsupported. The variability in the adherence of microorganisms even from the same species is probably too high to conclude on the efficacy of a material modification without testing numerous bacteria strains of the same species, also considering that the patient susceptibility may be influential too.

Results of our study also confirm that clinical studies require a large number of infection cases to consider the epidemiology and pathogenesis of different strains from each species under scrutiny. This is the only way to assess the potential risks. Furthermore, the isolated role of biomaterials and of components cannot be assessed without taking into account, at least statistically, the severity of the infection, the patient susceptibility, and the pathogenicity of the infecting microorganisms.

Of particular interest is the fact that our clinical series did not confirm basic experimental knowledge [13] about microorganisms being more adherent to polymers, which supposedly could present a higher risk of infection than metals. Furthermore, experimental infection may not mimic clinical variability because each particular bacterial strain used in any experimental study may show a high or a low adherence to a certain material or component while another strain of the same species may show different behaviour. This intraspecies variability is clearly established and is the reason for a recent trend that uses both collection and clinical strains of
microorganisms when performing a laboratory test of antibacterial activity of biomaterials [21], so as to achieve more realistic results.

This conclusion could have also relevant clinical consequences, as the polyethylene exchange performed in cases of early infection to decrease the microbiological load is unsupported. The substituted polyethylene component may even not hold adhered microorganisms, so unless all the components are revised and analyzed by sonication, it is impossible to know where most of the adhered bacteria are.

Therefore, large, multicentric series are required to definitely confirm the role, even secondary, of materials and components in the total joint infection. Despite its limitations, the major determinants in our series were not materials or components, but the patient undergoing a particular infection, and the pathogen microorganism.

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**FIGURE LEGENDS:**

Figure 1: Distribution of isolated bacteria per surface unit (CFU/mm$^2$) separated for hip and knee implants (mean with error bars showing SD).

Figure 2: Distribution of isolated bacteria per surface unit (CFU/mm$^2$) separated for type of component (mean with error bars showing SD).

Figure 3: Distribution of isolated bacteria per surface unit (CFU/mm$^2$) separated for type of biomaterial and implant (mean with error bars showing SD).

Figure 4: Femoral stem component (Versys, Zimmer, Warsaw IN, USA) result of surface measurement analysis.

Figure 5: Femoral component of a knee system (Scorpio, Stryker, Kalamazoo MI, USA) result of surface measurement analysis.
REFERENCES


