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(Article begins on next page)



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Reply to Dr. Esteban J. et al. letter to the Editor: Bacterial adherence to vitamin E UHMWPE. Considerations about *in vitro* studies.

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We thank Dr. Esteban J. *et al.* for careful reading of our manuscript and for their comments that we would like to discuss.

The recent article by Banche *et al.*, based on an interdisciplinary approach, focused on strong integration between different research groups that shared their experience and knowledge in orthopaedics, chemistry and microbiology, to elucidate the key role of biomaterial surface characteristics on microbial adhesion and biofilm formation.

In details, the primary aim of this study was to evaluate the influence of different chemical surface features of some prosthetic polyethylenes (standard UHMWPE, oxidized UHMWPE and vitamin E blended UHMWPE) on *S. epidermidis* adhesion.

The choice of *S. epidermidis* has been made taking into account the following factors:

i) coagulase-negative staphylococci, especially *S. epidermidis,* are opportunistic pathogens that mainly depend on the presence of indwelling foreign bodies to express their pathogenetic capacities in contrast to the more virulent *S. aureus* (1);

ii) *S. epidermidis* has been found to adhere more easily to polymers such as UHMWPE, whereas *S. aureus* more easily to metals (2);

iii) *S. epidermidis* is currently accepted as the most common cause of device related orthopaedic infections, according to recent scientific literature (3-5);

iiii) From 2000 to 2011 *S. epidermidis* has been isolated as frequently as *S. aureus* from BAI at the Department of Traumatology, Orthopaedics and Occupational Health (AO CTO/Maria Adelaide, Turin, Italy), where total joint replacements have been studied for many years;

In the study we tested a clinical *S. epidermidis* strain recently isolated from an orthopaedic implant infection (selected for its ability to establish clinically relevant infection on joint prosthesis) to compare its behavior with that of two laboratory ATCC *S. epidermidis* strains, chosen to allow the reproducibility of data. Our experimental studies required a well characterized model with the number of uncontrolled parameters as small as possible in order to highlight the potential different effects exerted by three prosthetic polyethylenes. In addition, during the course of the adhesion assays, all three bacterial strains were monitored for their virulence. Notwithstanding, there is a large number of literature papers that deal with this issue by testing only one bacterial strain (*S. epidermidis*), usually ATCC (3, 5-7).

As we demonstrated in this study, there is a correlation among different degree of hydrophilicity related to different protein adsorption, cholesterol and other molecules absorption, direct effect of vitamin E itself, even if present in very low concentration in the polyethylene, and *S. epidermidis* adhesion. The obtained results indicate that vitamin E blended UHMWPE reduced the adhesion of all the *S. epidermidis* strains

tested, both the clinical and the laboratory ones, and that the initial adhesion on inert surfaces is strain dependent.

As stated in the article, within the limitations of our study, these data must be considered tentative pending results from additional studies to provide a more thorough understanding process through the use of other organisms. Since all microorganisms can colonize an implant system, further studies are ongoing to evaluate the effect of these biomaterials with different chemical surface features also on the adhesion of other pathogens such as *S. aureus*, MRSA, *Pseudomonas aeruginosa*, *Candida albicans*, both clinical and laboratory strains to support a final conclusion of the real clinical value. To transfer into clinical practice experimentally obtained results, an *ex vivo* failed prosthetic component analysis will be necessary to correlate biomaterials surface with infection.

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