Evaluation of the effect of vitamin E doped UHMWPE on biofilm development and infection using an \textit{in vivo} experimental model

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Abstract. The aim of our study was the evaluation of an in vivo experimental model of implant-related septic arthritis. 5 x 10 mm strips out of a polyethylene sheet 500 microns thick were incubated with a 0.5 McFarland bacterial suspension of collection strains \textit{S. aureus} and \textit{S. epidermidis}. A surgical experimental model was prepared, implanting the samples in the subquadricipital articular space of the rabbit’s knee. After 7 days of survival, the knee joint was opened wide through the previous surgical approach and the polyethylene sample was retrieved. The UHMWPE samples were processed following a sonication and quantification protocol. Ten rabbits for each species were studied, five with each material. Negative controls (UHMWPE strips without attached bacteria) were also implanted in contralateral knees. \textit{S. aureus} colonies were detected only in three rabbits with non-treated UHMWPE and in two with vitamin E-doped UHMWPE. No differences in colony counts were observed for \textit{S. aureus}. No growth was detected for \textit{S. epidermidis}, although clinical signs of infection were detected in all animals with inoculated samples. The model was useful to evaluate the effect of modifications in biomaterials, although highly pathogenic bacteria are needed to obtain quantifiable data.

1. Introduction

Bacterial adherence to biomaterials is the origin of implant related infection, but limited information is available about this type of infections in experimental models.

Ultra-high molecular weight polyethylene (UHMWPE) is widely used in osteoarticular prostheses, particularly in knee and hip prostheses. Some studies demonstrate that UHMWPE is altered by the addition of Vitamin E (VE) to decrease material oxidation [1-5]. Potential effects of VE on bacterial adherence are currently being investigated, and the aim of this study was to evaluate the effect of UHMWPE-VE on biofilm development and infection in an \textit{in vivo} experimental model with rabbits.
2. Materials and methods

2.1. Collection strains and UHMWPE

*S. aureus* 15981 provided by Dr. Lasa [6] and *S. epidermidis* ATCC 35984 were used. The investigated material samples were 5 × 10 mm strips obtained from a UHMWPE virgin and commercial VE doped sheet 500 microns thick.

2.2. UHMWPE samples preparation

After overnight culture in Tryptic-soy broth, bacteria were harvested after 20 minute centrifugation at 3500g, and washed twice with sterile Phosphate Buffered Saline (PBS). The bacteria were then suspended and diluted in PBS to a concentration of $10^8$ colony-forming units (CFU)/ml. UHMWPE samples were placed in this bacterial suspension and incubated for 90 minutes at +37°C. After the incubation, specimens were rinsed twice with PBS proceeding with implantation.

2.3. Experimental model

A surgical experimental model was prepared, implanting the samples in the subquadricipital articular space of the rabbit’s knee as follows.

Under general anesthesia with IM Ketolar, a medial proximal parapatelar approach was performed, incising up to the quadricipital tendon. A blunt instrument was introduced from distal to proximal and, while maintaining the instrument as a guide, the sample was introduced and the joint was closed.

After 7 days the rabbits were slaughtered by pentobarbital overdose. Following the previous approach, the knee joint was opened wide and the polyethylene sample was retrieved with sterile forceps, and then introduced into a tube with a known amount of sterile PBS.

Samples were sonicated during 5 minutes and the number of bacteria was quantified by 1:10 serial plate counts.

Ten rabbits for each specie were studied, five with each material. Negative controls (UHMWPE strips without attached bacteria) were also used. Figure 1 shows the implantation process.

![Figure 1: Images of the arthrotomy. a) Opening of the articular space. b) UHMWPE sample. c) Introduction of the sample in the knee](image)

This protocol was repeated for *S. epidermidis* strain with the following modification: rabbits were slaughtered after 3, 7 and 14 days. After these periods, UHMWPE samples were processed as described above.
3. Results

CFU’s were only found in 5 out of 20 rabbits, all of them from the specie *S. aureus*, 3 in rabbits with non-treated UHMWPE and 2 with vitamin E doped UHMWPE. No differences in colony counts were observed for *S. aureus*.

No growth was detected for *S. epidermidis* in any experiment, although clinical signs of infection were detected in all animals with inoculated samples. Table 1 shows the colony counts results.

Table 1. Colony counts of UHMWPE with *S. aureus* after extraction from the rabbit’s knee

| NUMBER | UHMWPE | 10¹ | 10² | 10³ | CFU/ mL | TOTAL CFU |
|--------|--------|-----|-----|-----|---------|-----------|
| 1 V    | 10     | 1   | X   | 1 x 10⁴ | 3 x 10² |
| 2 V    | 58     | 4   | 3   | 4,9 x 10³ | 1,5 x 10⁴ |
| 3 V    | 10     | 1   | X   | 1 x 10⁴ | 3 x 10³ |
| 6 VE   | 77     | 8   | 2   | 7,8 x 10³ | 2,3 x 10⁴ |
| 10 VE  | 202    | 36  | 6   | 2,8 x 10⁴ | 8,4 x 10⁴ |

4. Discussion and Conclusions

Investigation of bacterial adherence related to implanted materials is a matter of increasing frequency and interest. *S. epidermidis* and *S. aureus* are the most frequent microorganisms involved in biomaterial-related infection, that always occur in relation to biofilm formation. This structure constitutes a protected mode for the bacteria to survive in a hostile environment.

Some factors, such as the surface of the implant, the local damage of the tissues when the devices are implanted or the systemic general condition of the individual may affect to the bacterial adhesion and thus, to the infection development [7]. For this reason it is important to realize more studies testing different kind of biomaterials.

A new experimental model was developed and evaluated that enables in-vivo testing of bacterial adherence to prosthetic materials [8]. *In vivo* studies are reported to simulate biomaterial infection. Even if the need to simulate human clinical conditions suggests using large animals, rabbit models are those most frequently found in the literature [7], because of their adequate size and fast responses.

Many strategies have been developed to prevent or reduce bacterial contamination in biomaterials. These include morphological or physical modifications. In this study, we use an *in vivo* model to test the effect of VE in UHMWPE. The model was useful to evaluate the effect of modifications in biomaterials, although highly pathogenic bacteria are needed to obtain quantifiable data. The absence of quantifiable results with *S. epidermidis* could be due to the differences between immune systems of humans and rabbits, even when a biofilm-producing strain was used and despite the fact of presence of clinical infection in the animals.

*S. aureus* consistently produced quantifiable joint infection when seeded in UHMWPE, but *S. epidermidis* produced clinical infection signs without objective, quantitative culture despite high inoculii and clinically aggressive strains.

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