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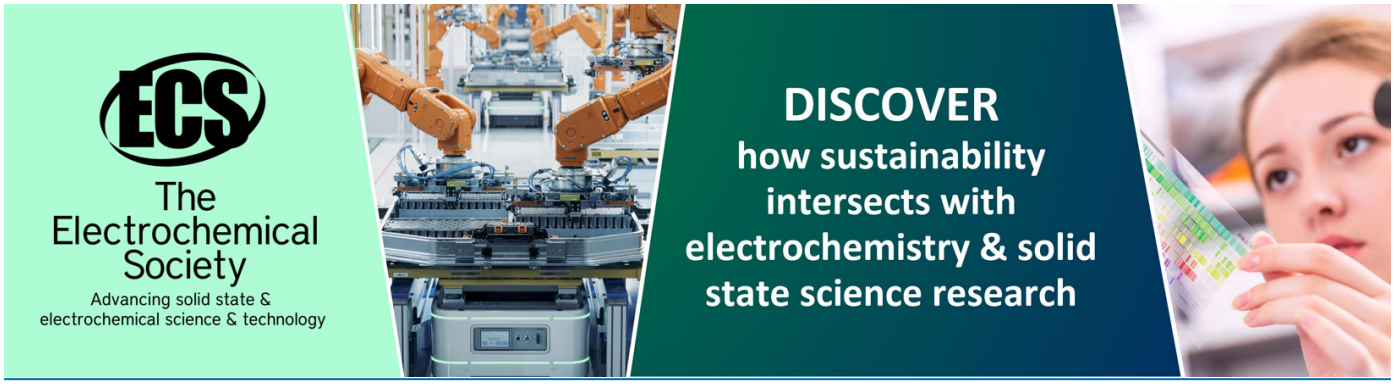

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Bacterial adherence on UHMWPE doped with Vitamin E: an *in vitro* study

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Abstract. Biomaterials may improve its capacity to resist bacterial adherence, and subsequent infection through material changes. Our aim was to test the bacterial adherence to vitamin E (VE) doped UHMWPE with *S. aureu*s and *S. epidermidis* (collection and clinical strains), compared to virgin material. Experimental UHMWPE with 3%, 0.4%, and commercial 0.1% VE concentration (1000 ppm) were tested. The biofilm-developing ability was used as a covariable. The collection strain of *S. aureus* showed significantly less adherence to the commercial VE UHMWPE (p=0.036) but the clinical strains did not significantly modified its adhesion to UHMWPE in presence of VE. The collection strain of *S. epidermidis* showed significantly less adherence to experimental UHMWPE with VE, independently of the concentration used (p=0.008). However, only 1 of the 4 clinical strains under study clearly confirmed these results in commercial VE polyethylene. Vitamin E doped UHMWPE affects the adherence of some *S. aureus* and *S. epidermidis* strains, independently of the concentration in use, but the results showed important intraspecies differences.

1. Introduction

Limited information is available regarding the adherence of microorganisms that cause orthopedic infection to biomaterials currently used in orthopedic implants. Yet it is important to understand the differences between orthopedic biomaterials to bacterial adherence, and to devise new methods for protecting these materials from such phenomenon.

Of particular interest is the polyethylene, today implanted as ultra-high molecular weight polyethylene (UHMWPE). Vitamin E (VE) has been recently incorporated to UHMWPE to decrease oxidation that may cause material degradation [1-5]. In vitro studies have also confirmed that VE remains within the material [6], thus the innovation has been accepted for the intended proposal, but it is not known if the susceptibility of microorganism adherence to polyethylene previously studied by

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our group [7] is altered by the addition of VE, which would be of significant clinical importance. Particularly, when potential effects of VE on chronic infection are currently being investigated, favoring the antibiotic action through cellular immunity enhancement [8] and cellular redox state [9].

The bacterial adhesion process is a complex series of physical and chemical interactions between the substratum and the microbe, and thus can be modified with changes in the biomaterial under risk. The use of VE in UHMWPE as a method to minimize material oxidization may also change the surface of the substratum and the bacterial adhesion process, thus limiting the extent of subsequent infection. With this hypothesis, we set the aims of the present study in the investigation of the adherence of the most frequently isolated bacterial species from orthopedic infections (*Staphylococcus aureus* and *Staphylococcus epidermidis*) in VE doped UHMWPE, compared to virgin material. For this, we investigated different VE concentrations with both collection and also with clinical strains isolated from orthopaedic infections.

2. Materials and methods

2.1. UHMWPE

The raw material used in the first part of the study was a compression molded sheet of GUR 1050 UHMWPE (Orthoplastic Ltd., Lancashire UK), from which 3 mm thick and 20 mm diameter discs were machined. All discs were grounded and polished up to an average surface roughness of R_a= 0.80 ± 0.05 μm using SiC papers. Vitamin E was introduced into UHMWPE by diffusion, soaking the disc in a bath of VE (α–tocoferol, Aldrich Chemicals). Two VE concentrations were prepared with the previous method, and gravimetric changes confirmed a VE content of 3 wt% and 0.4 wt% in the discs. All discs were sterilized with gas-plasma sterilization 10 days before the experiments were performed.

The second part of the study was performed in a commercial GUR 1020 UHMWPE with vitamin E, at a concentration of 1000 ppm (0.1%) obtained by blending (Meditech, Fort Wayne, Indiana, USA). Specimens of 228±13 μm thick and of 1cm^2 (1x1cm) area were cut from a sheet. The average roughness measured by a confocal microscope was 0.42±0.15 μm. Commercial GUR 1020 UHMWPE sheets without vitamin E were used as controls.

2.2. Bacterial strains

Collection strains of biofilm-producing *S. aureus* 15981, provided by Dr. Lasa [10], and *S. epidermidis* ATCC 35984 were used in the first set of experiments with different concentrations of VE (3% and 0,4%).

Clinical strains of both species were also tested in the second set of experiments performed using the commercial VE UHMWPE at a concentration of 0.1%. 5 *S. aureus* clinical strains and 4 *S. epidermidis* strains were used, besides the collection strains that were also used to confirm in this material the results of the first set of experiments. These clinical strains were isolated from cases of prosthetic joint infections using a sonication protocol previously described [11]. These strains were identified using conventional techniques. Quantification of the biofilm forming ability of the strains was tested and graded from 0 to 3 using the Stepanovic method [12].

2.3. Adherence study

Five samples of each experimental (3% and 0.4%) and commercial VE UHMWPE were tested for each bacterial strain. After overnight culture in Tryptic-soy broth, bacteria were harvested by 20 minute centrifugation at 3500g, and washed twice with sterile Phosphate Buffered Saline (PBS). Bacteria were then suspended and diluted in PBS to a concentration of 10^8 colony-forming units (CFU)/ml. The biomaterial samples were placed in this bacterial suspension and incubated for 90 minutes at +37°C. After the incubation, specimens were rinsed twice with PBS, and sonicated during 5 minutes in equal volume of PBS. The number of adhered bacteria was quantified by 1:10 serial plate counts.

SPSS 17.0 was used as statistical package (SPSS Inc., Chicago, IL).

3. Results

In the experiments with the collection strains, no significant differences were observed in the adherence of *S. aureus* to UHMWPE at any of the experimental concentrations used in the study (ANOVA, p=0.561). Mann-Whitney test showed non-significant differences in the adherence of *S. aureus* on UHMWPE with 3% VE (p=0.222) or with 0.4% VE (p=0.421), versus UHMWPE without VE. When the experiment was performed with the collection strain of *S. epidermidis*, significant differences in the adherence among series was detected with ANOVA (p=0.001), and post-hoc tests confirmed that differences were between control and VE doped material. Mann-Whitney test showed that the difference between control and UHMWPE with 3% VE concentration was significant (p=0.008), and that the difference stood when the VE concentration was lowered to 0.4% (p=0.008), as seen in Figure 1.

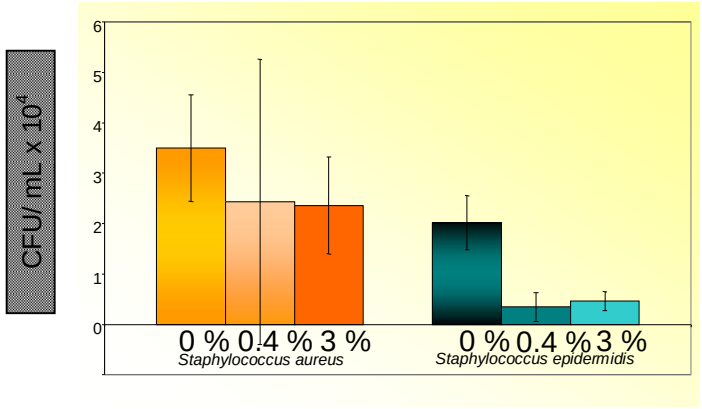

Figure 1: CFU (error bars, SD) quantified after adherence on UHMWPE without VE, with 0.4 and 3% VE, for collection *S. aureus* and collection *S. epidermidis* (n=5).

When clinically isolated strains were studied on the 0.1% VE UHMWPE, no significant differences were obtained comparing in a paired t-test the culture counts of all *S. aureus* strains (p=0.107) and of *S. epidermidis* strains (p=0.252) on virgin versus VE-UHMWPE. However, the results were highly variable among individual strains. One factor ANOVA and Kruskal-Wallis tests, used in view of the n=5 repetitions of the experiment per strain, showed that culture counts significantly differ among strains of *S aureus* in virgin (p=0.010) and VE (p=0.014), while with *S. epidermidis*, differences were significant in VE (p=0.003) among strains. When each strain was investigated in both materials, Mann-Whitney test showed that *S. aureus* clinical strains did not significantly decrease its adherence to VE (table 2), but it did in the collection strain (p=0.036). Mann-Whitney tests in *S. epidermidis* collection strain did not significantly modify (p=0.841) its adherence when in the presence of VE UHMWPE, but it did in of the 2 of the 4 clinical strains under investigation (table 1), although one strain in the decrease and one with an increase.

Table 1: Comparison of adherence per clinical strain, virgin UHMWPE versus commercial VE UHMWPE (Mann-Whitney test, p>0.05).

Clinical strain	Microorganism	Culture from sonicated material after adherence to virgin UHMWPE (mean±SD)	Culture from sonicated material after adherence to Vitamin E UHMWPE (mean±SD)	Significance
1	*S. aureus*	4.33±4,58	1.23±0.39	0.114
2	*S. aureus*	0.75±0.40	0.74±0.27	0.690
4	*S. aureus*	3.59±4.25	1.32±0.51	0.200
61	*S. aureus*	1.39±0.81	1.28±0.81	0.886
95	*S. aureus*	0.08±0.04	0.27±0.38	0.343
Collection strain	*S. aureus*	1.11±0.34	0.12±0.10	**0.036***
53	*S. epidermidis*	0.38±0.36	3.20±1.38	**0.008***
55	*S. epidermidis*	1.21±0.68	5.61±6.67	0.886
74	*S. epidermidis*	1.31±1.07	1.58±0.74	0.686
101	*S. epidermidis*	2.65±1.51	0.07±0.05	**0.008***
Collection strain	*S. epidermidis*	0.64±0.17	0.75±0.56	0.841

The investigation on the covariable established by the Stepanovic grading of biofilm-forming ability showed that this grade did not influence changes in adherence of *S. aureus* strains on VE UHMWPE (p=0.305, Kruskal-Wallis test) or virgin material (p=0.133, Kruskal-Wallis test), but did in the case of *S. epidermidis* on VE UHMWPE (p=0.002, Kruskal-Wallis test) and virgin material (p=0.020, Kruskal-Wallis test). When the ANOVA test with Bonferroni post hoc was used to clarify the effect of multiple comparisons of culture counts among Stepanovic graded strains, significant differences were found in the adherence on virgin UHMWPE and VE UHMWPE as shown in table 2 and 3.

Table 2: Descriptive culture counts of *S epidermidis* clinical strains grouped by Stepanovic grade (mean+-SD).

UHMWPE	Stepanovic grade	n	Mean	SD
Virgin	0	5	2.64700	1.515188
	1	8	1.26375	0.835394
	2	5	0.38480	0.357527
Vitamin E	0	5	0.0700	0.04899
	1	8	1.8771	1.29404
	2	5	3.2020	1.37905

Table 3: Comparison of *S. epidermidis* culture counts of strains with different Stepanovic grading for the 2 studied materials (ANOVA with Bonferroni post hoc test, significance with p>0.0125).

Polyethylene	Compared Stepanovic grades	Bonferroni significance (p>0.0125)
Virgin UHMWPE	0 vs 1	p=0.079
	1 vs 2	p=0.416
	0 vs. 2	p=0.007*
Vitamin E UHMWPE	0 vs 1	p=0.041
	1 vs 2	p=0.106
	0 vs. 2	p=0.002*

4. Discussion and Conclusions

Significant efforts have been placed in the control of UHMWPE oxidation as a means to lower material and implant failure in total joint replacements. Vitamin E doped material is one of these proposals. The burden of total joint replacement failure is also reltated to infection. Infection related to orthopaedic implants frequently develops from biofilm formation after bacterial colonization of the biomaterial. In this study, VE affects the adherence of *S. epidermidis* on UHMWPE but not that of *S. aureus* on collection strains. This finding stood with different concentration of VE, and there was no appparent relationship to VE dosage.

The mechanism by which VE may affect the bacterial adherence to UHMWPE is currently unknown.

The relative adherence to a certain material thus may be associated to strains differences.

As we demonstrated in this study, the evaluation of clinical strains is mandatory to ascertain the consistency of results in adherence studies. In this sense, we tested several clinical strains randomly chosen from the bank of clinical strains isolated from patients with orthopaedic infection. We found significant variability among these clinical strains, and the VE effect detected with collection *S. epidermidis* strain in the experimental setting was not confirmed in the commercial VE UHMWPE but in one of the particular 4 clinical strains that were used in our study. On the opposite, a detected effect by VE with collection *S. aureus* strain was insignificant in the experiments but definitely observed in the commercial VE polyethylene, while the selected 5 clinical strains failed to confirm a decrease adherence to VE UHMWPE.

Stepanovic grading was used to search for a relationship between the capability of forming biofilm as a severity index of the potential pathogenic effect of the strain and the adherence to UHMWPE. A paradoxal effect was found with virgin UHMWPE, as less adherence was observed for higher Stepanovic grades, while high adherence was found in higher Stepanovic grades on VE UHMWPE. Other studies of biofilm formation on UHMWPE doped with vitamin E are on the way to follow this issue.

Potential effects of VE on infection are currently being investigated, but mostly directed towards immunomodulation in chronic diseases [8]. Also, a cellular redox state change has been invocated as a beneficial factor of VE [9]. As adherence is modulated by surface redox state, this may affect adherence mechanisms on certain microorganism strains, yet unclear. We were able to confirm that incorporating VE produces changes in the surface compared with the virgin UHMWPE [7]. Despite the appealing of this hypothesis, we were only capable of confirming that VE significantly affected the adherence in some clinical and collection strains, but not in others, and the clues to understand why some strains are more susceptible to the VE than others are not yet clarified. Nevertheless, VE incorporates a biological surplus in the modified material that is effective in reducing the adherence of some strains of *S. aureus* (collection strain in our study) and *S. epidermidis* (collection in one set of

experiments, clinical strain in the other set) despite the absence of specific antibacterial effect of vitamin E tested by microdilution (data not shown).

We think this finding could introduce an added value to vitamin E doped UHMWPE, although this is an *in vitro* study on the very early stage of the colonization process. The use of clinical strains is mandatory in these studies, because of intrinsic intra and interspecies variability among different bacterial genera, but the limitation is that the number of clinical strain to be tested to clarify this issue could be extremely high and out of the capabilities of an experimental research study.

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