BACTERIAL ADHESION TO IMPLANTABLE MATERIALS

LLINOS G HARRIS, R. GEOFF RICHARDS

AO RESEARCH INSTITUTE, DAVOS, SWITZERLAND

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Introduction

Staphylococcus aureus and *S. epidermidis* are human pathogens that colonise implant surfaces. They are of increasing importance due to the rise in antibiotic resistance [1]. *S. aureus* is one of the main causes of metal-biomaterial, bone joint and soft tissue infections [2], and is distinct from *S. epidermidis* which is an opportunistic bacteria, associated with catheters and other indwelling medical devices [3]. *S. aureus* and *S. epidermidis* are both capable of forming biofilms which can be difficult to clinically treat because the bacteria in the interior of the biofilm are protected from phagocytosis and antibiotics [4], hence the need to prevent bacterial adhesion to implants. This may be possible by modifying the topography/chemistry of the implant surface, or coating it with an antimicrobial/protein resistant coating. Here we describe methods for the visualisation and quantification of *S. aureus* and *S. epidermidis* adhering to different implantable biomaterials.

Methods

To visualise S. aureus and S. epidermidis adherence on different surfaces, bacteria were cultured on the various modified biomaterial surfaces in brain heart infusion broth (BHI) at 37°C over several time periods, then fixed with 2.5% glutaraldehyde in buffer, post-stained with 1% osmium tetroxide, dehydrated, critical point dried, coated with Au/Pd, and visualised with a scanning electron microscope using a backscattered electron detector [5]. To quantify the amount of bacterial adhesion, adherent bacteria were stained with fluorescent 5-cyano,2-ditolyl tetrazolium chloride (CTC), and visualised with a Zeiss Epifluorescence microscope fitted with an Axiocam camera [6]. The density of live bacteria on the surfaces in each image was counted using KS400 software. On surfaces that auto-fluoresce, adherent S. aureus and S. epidermidis were detached by sonication in Tween 80, then stained with a live/dead assay (Molecular Probes). The amount of bacteria present were counted using a Partec PAS flow cytometer.

Results

SEM images showed variations in the adhesion of *S. aureus* and *S. epidermidis* to the surfaces (FIG.1). No differences in the amount of S. aureus adhesion were observed on standard titanium (TS), electropolished titanium (TE) and TS coated with nitrogen ions (TIG) surfaces, slightly more were found on TS coated with polymer for promoting cell adhesion (TAST), but few were found on the TS coated with hyaluronate acid (THY) (FIG. 2). In comparison to the uncoated titanium surface (TiS), few bacteria were seen on the PLL-g-PEG (PEG) and PLL-g-PEG functionalised with RGD surfaces (PEG-RGD) (FIG. 2b).

Flow cytometry results quantified the difference in the amount of *S. aureus* and *S. epidermidis* adhering to vari-

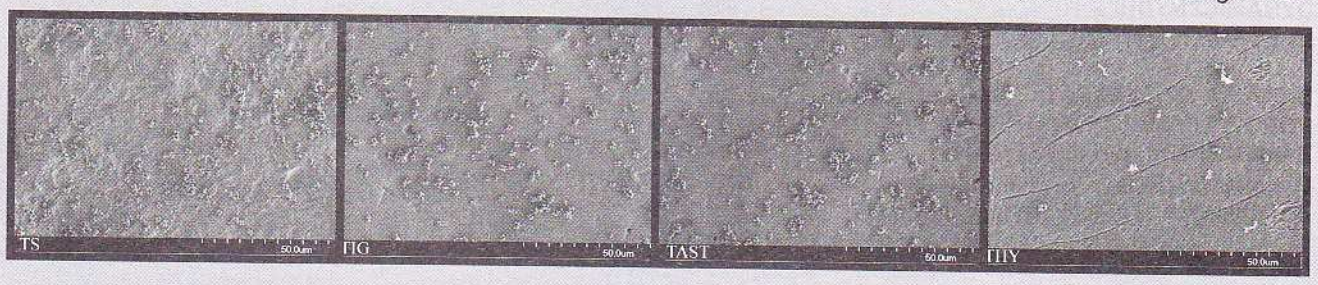

FIG. 1. SEM images of *S. aureus* adhering to different materials.

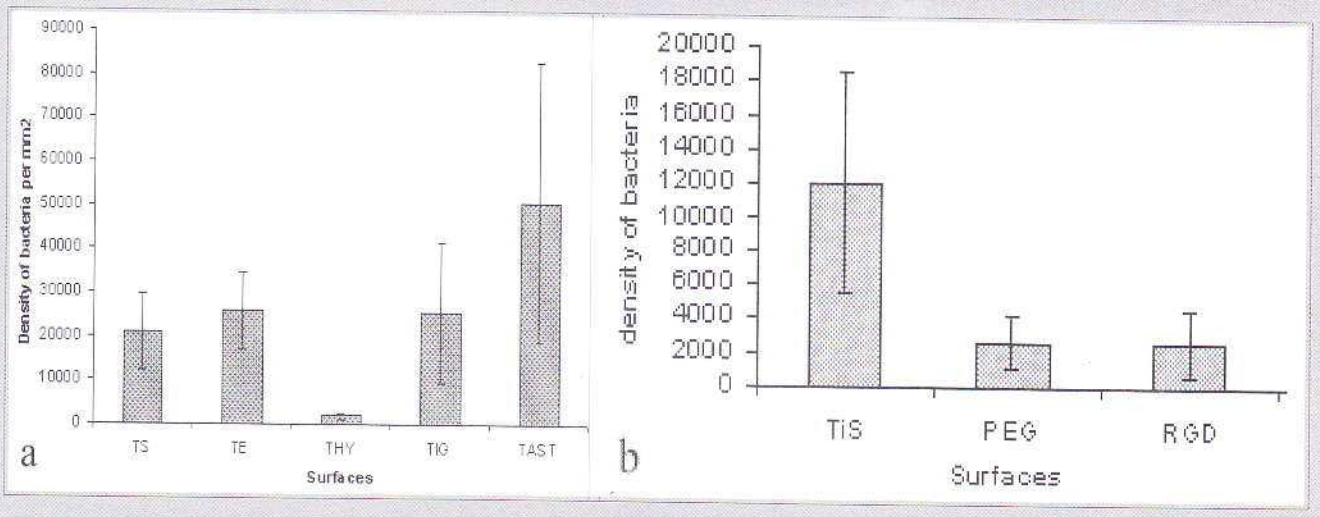

FIG. 2. Graphs showing the density of bacteria on different surfaces after 1h of culturing. a) on TS, TE, TIG, TAST and THY; b) TiS, PEG and PEG-RGD.

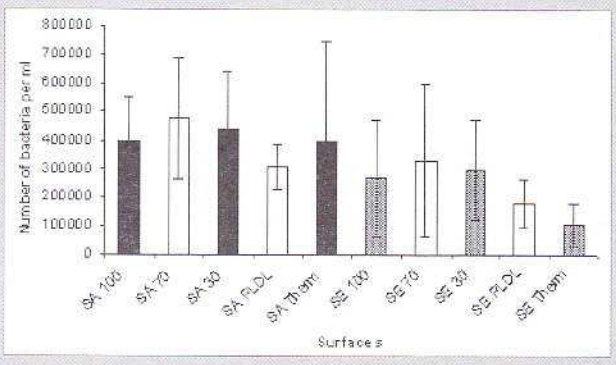

FIG. 3. Graph showing flow cytometry results from SA (*S. aureus*), and SE (*S. epidermidis*) adhering to different polyurethanes.

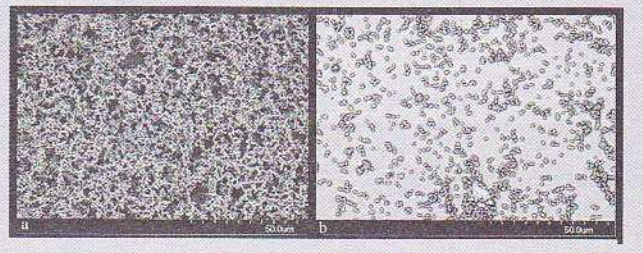

FIG. 4. SEM images of a) *S. aureus* and b) *S. epidermidis* adhering to 70% polyurethane.

ous surfaces. The example shown in FIGURE 3 confirmed SEM observations (FIG.4), that more *S. aureus* adhered to the surfaces than *S. epidermidis*.

Discussion and conclusions

These results show that different methods can be used to study the adhesion of bacteria to biomaterials in vitro. SEM is useful for morphology and general observations, and depending on the material (polymers tend to auto-fluoresce), either fluorescence microscopy or flow cytometry can be used to quantify the amount of adherent bacteria.

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THE RADIOIMMUNO-ASSAY OF CORTISOLE LEVEL IN MIXED SALIVA FROM THE PATIENTS WITH MULTIPLE DENTAL CARIES

I.O. Pohodenko-Chudakova, Y.M. Dosin, A.A. Varno

Bellorussian State Medical University
Pushkina av. 33 - 239, 220092 Minsk, Belarus

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Caries is known to be accompanied by the metabolism impairment in a human organism. These changes involve the oral liquid medium, which is mixed saliva. A great number of articles are devoted to the protective role of the oral fluid, its participation in oral metabolism. However little attention is paid to the study of cortisole level in mixed saliva from patients with different degrees of caries.

The aim

of presented article was to study of cortisole level in mixed saliva from patients with different degrees of caries.

Materials and methods

For this purpose we have studied 3 groups of patients from 15 to 25 years of age. The samples of saliva for research were collected in the morning time, before breakfast in disposable sterile tubes.
Before testing, samples were stored in liquid nitrogen at temperature - 196°C. The hormone levelsin oral fluid was determined by using cortisole marked with Iodine 125 (Steron-C-125 I) in the detector of Gamma-camera measuring the speed of sedimentation in each sample in 1minute. After that we determined the cortisole level in nMol/l.

Results

Analysis of the results of our research shows that there are considerable differences in concentration of cortisole in saliva from people with low and high intensity of caries ($p < 0,01$) and low and middle intensity of the process ($p < 0,05$). Differences between middle and high caries intensity groups weren't reliable ($p < 0,05$).

Conclusion

On the basis of our research we can say that change of cortisole level in mixed saliva reflects growth of cariesogenic situation in the oral cavity. Consequently, this test can be used for diagnostic purposes and for determination of the effectiveness of treatment and preventive measures.