ABSTRACT:

Background: One of the major drawbacks in the use of biomedical materials is the occurrence of biomaterial-centered infections. Adhesion of microorganisms to an implant is mediated by their physico-chemical surface properties and the properties of the biomaterial surface itself. Subsequent surface growth of the microorganisms will lead to a mature biofilm and infection, which is difficult to eradicate by antibiotics.

Objective: The purpose of this research is to examine the adhesion in the combined cultivation of Staphylococcus aureus and the Lactobacillus plantarum probiotic bacterium on the surface of different metals (copper, aluminium, low-carbon steel, and zinc).

Methods: The precise weighing (with an allowance of 0.0001 g) of the metal plates before and after the treatment found a minimum negative change in their weight, which may be caused by reduction resulting from corrosion processes, on one hand, or growth because of the forming of a biofilm, on the other. The structure of the layer over the metal plates was analysed by SEM (scanning electron microscopy) JSM 5510.

Results: The thinnest biofilm for both bacteria was registered on the surface of the copper plate. When a combined culture is used on the surface of the aluminium and the steel plates, the pathogenic bacterium is adhered predominantly. On the zinc plate it is only the probiotic bacterium that adheres.

Conclusion: This is an initial research on this problem of significance for the doctors and it is about to be further examined.

Keywords: adhesion, biofilm, Staphylococcus aureus, Lactobacillus plantarum

INTRODUCTION:

Foreign materials are used more and more in modern medicine after trauma, oncological surgery or wear to replace, support or restore human body function, for example in hip prostheses, prosthetic heart valves or catheters [1]. The extended use of these materials, usually referred to as biomaterials, has some major drawbacks. One of these is the possible occurrence of biomaterial-centered infections (BCI) [2]. The incidence of this type of infections varies from 4% for hip prostheses [3] to 100% for urinary tract catheters after 3 weeks use [4]. Adhesion of microorganisms to an implant is mediated by their physico-chemical surface properties and the properties of the biomaterial surface itself. Subsequent surface growth of the microorganisms will lead to a mature biofilm and infection, which is difficult to eradicate by antibiotics. Treatment of BCI is complicated, as microorganisms in a biofilm are more resistant to antibiotics [5] than their planktonic counterparts [6]. As a consequence and if possible, the only remedy is removal of the infected implant at the expense of considerable costs and patient’s suffering. A more convenient way to deal with this problem is to prevent the development of an infectious biofilm on the biomaterial surface. To achieve this, a thorough understanding of how these biofilms develop is necessary [1]. The search for biomaterials that are able to provide for the optimal resistance to the infection can be based only on the deep understanding of the interactions between bacteria and biomaterials.

The purpose of this research is to examine the adhesion in vitro in the combined cultivation of Staphylococcus aureus and the Lactobacillus plantarum probiotic bacterium on the surface of different metals (copper, aluminium, low-carbon steel, and zinc).

MATERIALS AND METHODS:

Test Microorganisms

Staphylococcus aureus 745 were obtained from the Collection of the Department of General and Applied Microbiology, Sofia University. The isolate were checked for purity and maintained in slant of Nutrient agar. Nutrient Agar (Biolife 272-20128, Milano, Italia) was the medium used as the growth medium for the microbe.

The Lactobacillus plantarum strain was isolated from commercial probiotic product. The strain cultivated in media of MRS (de Mann Rogosa Sharpe, Biolife 272-20128, Milano, Italia) in composition, per liter: glucose – 20.0; Tween 80 - 1; pepton from casein – 10.0; meat extract - 8.0; yeast extract - 4.0; K2HPO4 - 2.0; sodium acetate - 5.0; ammonium citrate - 2.0; MgSO4.7H2O - 0.2 and MnSO4 - 0.05 g/L. The pH of media was adjusted to 6.5 with 1M NaOH. The basic media was sterilized by autoclaving at 121°C for 20 min.
Before the assays, the strains *L. plantarum* and *S. aureus* 745 were twice pre-cultured in MRS broth and Nutrient broth respectively, for 24 h at 37° C. Exponential cultures in broths were used as inoculum for the adhesion analysis.

**Preparation of the metal samples**

The steel plates made of copper, aluminium, low-carbon steel, and zinc are weighed with an allowance of 0,0001 g with an assay-balance. The precise weighing (with an allowance of 0,0001 g) of the metal plates before and after the treatment found a minimum negative change in their weight, which may be caused by reduction resulting from corrosion processes, on one hand, or growth because of the forming of a biofilm, on the other. They are put sterilely in a liquid ambient which contains a mixture of *L. plantarum* and *S. aureus* 745 in a proportion 1:1. The samples were incubated at 37° C for 24 h. The structure of the layer over the metal plates was analysed by SEM (scanning electron microscopy) JSM 5510.

All experiments were performed in triplicate.

**RESULTS:**

The results obtained from the SEM analysis of the adhesion ability of the tested microorganisms on the different metals are shown in figure 1. The thinnest biofilm for both bacteria was registered on the surface of the copper plate (fig. 1B). When a combined culture is used on the surface of the aluminium and the steel plates, the pathogenic bacteria is adhered predominantly (fig.1 A and C). On the zinc plate it is only the probiotic bacterium that adheres (fig.1 D).

![Fig. 1. SEM of the tested samples.](http://www.journal-imab-bg.org)

1A) low-carbon steel; 1B) copper; 1C) aluminium; 1D) zinc.
Van Loosdrecht et al. [7] concluded that adhesion of bacteria does not directly influence their metabolism and growth yield. Changes in growth rate due to adhesion of bacteria were suggested to be mainly the result of changes in nutrient availability [1]. Depending on the amount of adsorbed nutrients and whether adsorption is easily reversed, growth rates of adhering bacteria can be decreased or increased with respect to the growth of their planktonic counterparts. Probably the materials differences played a role. S. aureus grew faster on the metal, while S. epidermidis grew faster on polymeric biomaterials [1].

**DISCUSSION:**
When microorganisms have reached the biomaterial surface, initial microbial adhesion can occur. Microbial adhesion is mediated by specific interactions between cell surface structures and specific molecular groups on the substratum surface [8], or when viewed from an overall, physico-chemical view-point by non-specific interaction forces, including Lifshitz-Van der Waals forces, electrostatic forces, acid-base interactions and Brownian motion forces [9].

After adhesion to biomaterials most microorganisms start secreting slime and embed themselves in a slime layer, the glycocalix, which is an important virulence factor for BCI and which explains the extraordinary prevalence of slime producing *S. epidermidis* in BCI [8]. The glycocalix provides protection against humoral and excreted cellular immune components, as these can not readily diffuse through the slime layer [5] and once a glycocalix has formed a BCI with all its complications, including ultimately removal of the implant, seems almost inevitable.

In previous publications we have shown that lactic acid bacteria are capable of forming a biofilm by producing exopolysaccharides which protect the metals from corrosion [10, 11, 12]. Moreover, we have shown that some of the end products of the fermentation process are also able to form a protective layer on the metal surfaces [13]. However, why it is only on the zinc plate that a biofilm of the beneficial bacteria is formed is a question difficult to answer at this stage. In our opinion, differences are also likely to appear in the adhesion process under *in vitro* and *in vivo* conditions because other processes are going to have an impact in the living organism, too. Kristopher P. et.al. [14] concluded that hydrophobic and photoinduced superhydrophilic surface coatings both have potential as a means of reducing microbial fouling of surfaces. According to the updated paradigm for biocompatibility, as redrawn by Williams [15], a biomaterial should perform its designed function eliciting the most appropriate tissue response. This performance is especially expected now that biomaterials need to be able to act as sophisticated gene/cell/drug delivery systems, and are more and more turned towards biotechnology and tissue engineering applications. Nevertheless, in the vast majority of clinical implantations, the first requirement of a material’s biocompatibility is that, whatever the desired function, the material shall not induce any adverse effects in the patient, “just as the first principle of Hippocrates was that the doctor should do no harm” Biomaterial science has greatly progressed in the achievement of a safe biocompatibility, free from damage to neighbouring tissues. Unfortunately, as Gristina warned ever since 1987, a major drawback to implanted devices remains “the possibility of bacterial adhesion to biomaterials, which causes biomaterial-centred infection” [15].

**CONCLUSION/S/:**
The various metabolic ways and the various end metabolic products of the two types of bacteria, *Staphylococcus aureus* and *Lactobacillus plantarum*, could explain to a certain extent the different biofilms formed on the different metal surfaces. Different types of complex compounds are probably formed between the secreted exopolysaccharides or the end metabolic products of the two types of bacteria, as Gristina warned ever since 1987, a major drawback to implanted devices remains “the possibility of bacterial adhesion to biomaterials, which causes biomaterial-centred infection” [15].

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