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Material influence on biocontamination level and adhering cell physiology

Influence des matériaux sur le niveau de biocontamination et la physiologie des cellules adhérentes

Audrey Allion[1], Jean-Marie Herry[2] and Marie-Nöelle Bellon-Fontaine[2]

[1] *APERAM Research Center, 62330 Isbergues, France*
[2] *INRA-AgroParisTech, Institut MICALIS, UMR 1319, équipe «Bioadhésion et hygiène des matériaux», 25 avenue de la République, 91300 Massy, France*

Abstract. In most environments, association with a surface in a structure known as a biofilm is the prevailing microbial lifestyle. Several factors may influence the biofilm formation *e.g.* nutrients, temperature, flow velocity, initial microflora and the nature of materials. Considering the biocontamination mechanism described in four steps, the initial adhesion is a key element in the biocontamination phenomenon and the substratum is of major concern in controlling bacterial adhesion. Stainless steel is well used in numerous markets because of its high cleanability and corrosion resistance properties. However, other materials are put forward by focusing on properties which differentiate them from those of stainless steel. Thereby, to select the material best suited to the problem, there should have data on their aptitude for biocontamination as well as adhesion impact on cell physiology. For all materials, the ratio of dead adhering cells is lower than 55%. The results obtained show that cell injury is not higher on material known to be bactericidal than on other ones.

Résumé. Dans la plupart des environnements, les microorganismes vivent préférentiellement au sein de biofilms. De nombreux facteurs influencent leur formation *i.e.* les nutriments, la température, le régime du fluide environnent, la microflore et les matériaux. Dans le mécanisme de biocontamination, décrit en quatre étapes successives, l'adhésion initiale est un élément clé de la bioadhésion et les matériaux un élément majeur pour son contrôle. L'acier inoxydable est très utilisé dans de nombreux secteurs d'activité pour sa bonne nettoyabilité et son excellente résistance à la corrosion. Pour se différencier, certains matériaux mettent en avant d'autres propriétés. Ainsi, la sélection du matériau le mieux adapté à un problème donné nécessite de connaitre son aptitude à la biocontamination ainsi que son impact sur la physiologie des microorganismes. Pour tous les matériaux testés, la mortalité des bactéries adhérentes est inférieure à 55 %. Les résultats obtenus ont montré qu'un matériau dit antimicrobien n'induit pas plus de cellules endommagées comparativement aux autres matériaux.

INTRODUCTION

Fouling of surfaces by microorganisms is a widespread phenomenon in industries, and also in medical appliances. When biofouling is due to soiling microorganisms, the product quality will be altered leading to economical losses. If it involves pathogens, it can lead to public health problems such as toxi-infection or nosocomial infections [1]. The biocontamination mechanism is described in four steps [2]:

1 – **transport** of microorganisms into close contact with the surfaces by sedimentation, Brownian movement, flow shear [1], microbial motility [3].
2 – **initial adhesion** depending on the net sum of attractive or repulsive physico-chemical forces generated between the cells and the surfaces [4,5], [6,7].
3 – **consolidation of the adhesion** with more specific molecular interactions between the micro-organism and the substrata [6].
4 – **surface colonisation** is the result of the cell multiplication. Nutrients adsorbed onto the solid/liquid interface permit the microbial growth [5]. Then, adhering bacteria synthesise exopolysaccharides that entrappe micro-organisms and nutrients in a matrix [8]. This matrix and the cells embedded in it form a biofilm. [3].

Thus, the initial adhesion is a key element in the biocontamination phenomenon and the substratum is of major concern in controlling bacterial adhesion.

Stainless steel is used in numerous markets because of its properties such as high cleanability and high corrosion resistance. However, other materials are put forward by focusing on properties which differentiate them from those of stainless steel. So, the aim of this study is to compare the behavior of stainless steel, in terms of biocontamination level and impact on the physiology of adhering bacteria, with competitors.

MATERIAL & METHODS

Bacterial strain

Staphylococcus aureus CIP 53.154, was grown at 37 °C in static conditions until the stationary phase culture in TSB (Bio-Rad, France). Bacteria were harvested by centrifugation (7000 g, 10 min.), washed and resuspended in NaCl 0.15 M (10^8cfu/ml) for the experiments.

Tested materials

The study has been carried out using two ferritic (K41 and K44) and two austenitic (304L and 316L) stainless steel grades as well as copper, aluminium and zinc.

Prior to any testing, surfaces were soaked for 10 min at 50 °C in a 2% (v/v) RBS 35 (Société des traitements chimiques de surface, France) and rinsed 5 times for 5 min in distilled water at 50 °C and 5 times for 5 min in distilled water at room temperature. Samples are kept in sterile Petri dishes for 24h before experimentations.

Solid surfaces characterizations

The surface wettability was determined using the contact angle measurement by the sessile drop method. The surface free energy, γ_S, was determined from contact angle measurements using the following equation [9]:

$$\gamma_L(\cos\theta + 1) = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right).$$

Adhesion experiments

Substrata chips were immersed in the bacterial suspension in static conditions at room temperature for 1 h. Non-sticking cells were removed by five rinses with NaCl 0.15 M.

Biocontamination assessment

Adherent cells were labelled with a solution of two fluorochromes (DAPI and Sytox Green®, Molecular Probes, USA). Blue-labelled (DAPI) cells represent total adherent bacteria whereas green (SYTOX GREEN®) labelled bacteria were considered as dead [10]. The fouled samples were observed with an epifluorescence microscope. For each observed field, numbers of total and dead cells were determined by image analysis (Analysis, USA) and expressed as cells/cm^2.

RESULTS

The measured water contact angles, the Lifshitz- van der Waals (γ^{LW}), Lewis acid-base (γ^{AB}) and Lewis-base (γ^-) components of the surface free energy are summarized in Table 1.

The measured WCA are comprised between 59 and 74.3° for stainless steel grades. Aluminum exhibits a strong hydrophilic character with a WCA of 12°. Otherwise, copper and zinc appear hydrophobic (WCA above 80°). For all material, the free surface energy is mainly due to the Lifshitz- van der Waals surface component. This value is comprised between 33 and 42.8 mJ/m^2. Most of the materials exhibit polar component, the lowest values are calculated for copper, zinc and K44.

Figure 1 represents the surface biocontamination (expressed as %) deduced from total adhering bacteria counts for all tested materials. Significant different results are observed ($P < 0.05$). The lowest biocontamination is obtained for stainless steel grades and highest is measured on aluminium, copper and zinc.

As *S. aureus* presents a strong Lewis-base (γ^-) character, the lower the surface Lewis-base component, the higher the biocontamination level.

Figure 2 represents the total and the injured adhering cells of *S. aureus* on all materials. On all materials, injured adhering bacteria represent less than 55% of the total adhering cells. These results show than none of the tested material has bactericidal property against adhering cells of *S. aureus*.

Table 1. Water wettability & free surface energy componants.

	Water contact Angle (WCA°)	Free surface energy and its components (mJ/m^1)		
		γ^{LW}	γ^{AB}	γ^-
304	69.6	36.7	2.7	14.1
316	59.6	40.4	3.6	21.0
K41	69.3	33.7	3.1	16.1
K44	74.3	36.0	0.0	16.9
Cu	93.5	36.8	0.1	3.6
Al	12.8	40.6	2.6	67.0
Zn	81.4	36.7	0.2	17.4

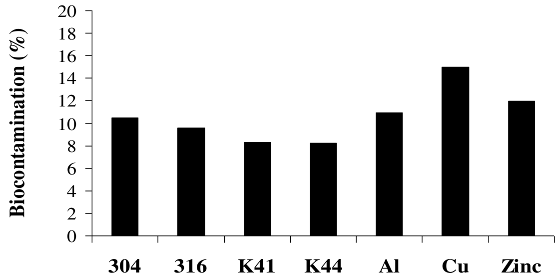

Figure 1. Material surface biocontamination (expressed as %) by S. aureus 53.154.

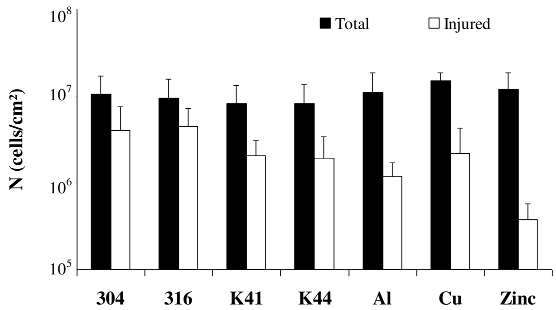

Figure 2. Total and injured adhering bacteria on materials under study.

CONCLUSIONS

The lowest biocontamination is obtained for stainless steel grades while highest is measured on aluminium, copper and zinc. On all materials, injured adhering bacteria represent less than 55% of the total adhering cells. These results show that the tested material don't have bactericidal property against adhering cells of *S. aureus* CIP 53.154.

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