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The effect of surface properties of polycrystalline, single phase metal coatings on bacterial retention

Kathryn A. Whitehead [a,b,*], Sebastien Olivier [c,1], Paul S. Benson [a], Nils Arneborg [d], Joanna Verran [e], Peter Kelly [b]

[a] School of Health Care Science, Manchester Metropolitan University, Chester St, Manchester M1 5GD, UK
[b] Surface Engineering Group, Manchester Metropolitan University, Chester St, Manchester M1 5GD, UK
[c] Ecole Nationale Superieure des Ingnieurs en Arts Chimiques et Technologies, 118 Route de Narbonne, 31077 Toulouse, Cedex 4, France
[d] Department of Food Science, Food Microbiology, Faculty of Science, University of Copenhagen, Denmark
[e] School of Research Enterprise and Development, Manchester Metropolitan University, Chester St, Manchester M1 5GD, UK

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ABSTRACT

In the food industry microbial contamination of surfaces can result in product spoilage which may lead to potential health problems of the consumer. Surface properties can have a substantial effect on microbial retention. The surface characteristics of chemically different coatings (Cu, Ti, Mo, Ag, Fe) were defined using white light profilometry (micro-topography and surface features), atomic force microscopy (nano-topography) and physicochemical measurements. The Ag coating had the greatest topography measurements and Fe and Mo the least. Mo was the most hydrophobic coating (lowest γ_{AB}, γ^+, γ^-) whilst Ag was the most hydrophilic (greatest γ_{AB}, γ^+, γ^-). The physicochemical results for the Fe, Ti and Cu coatings were found to lie between those of the Ag and Mo coatings. Microbiological retention assays were carried out using *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* in order to determine how surface properties influenced microbial retention. It was found that surface chemistry had an effect on microbial retention, whereas the shape of the surface features and nano-topography did not. *L. monocytogenes* and *S. aureus* retention to the surfaces were mostly affected by surface micro-topography, whereas retention of *E. coli* to the coatings was mostly affected by the coating physicochemistry. There was no trend observed between the bacterial cell surface physicochemistry and the coating physicochemistry.

This work highlights that different surface properties may be linked to factors affecting microbial retention hence, the use of surface chemistry, topography or physicochemical factors alone to describe microbial retention to a surface is no longer adequate. Moreover, the effects of surface parameters on microbial retention should be considered individually for each bacterial genus.

1. Introduction

A major concern in the food processing industry is to ensure that food produce does not become spoiled or cause disease in humans as a result of bacterial contamination. Bacteria naturally occur in raw materials, but may (re)contaminate food products due to their residing in food processing equipment (Carrasco et al., 2012; Skovager et al., 2012). Stainless steel, a Fe alloy usually containing Cr in excess of 11% but less than 30% (Adams, 1983) is regularly used in the manufacturer of food processing equipment since it is relatively easy to clean, mechanically strong, is relatively easy to fabricate and is corrosion resistant. However, it has been shown that even with good manufacturing processes that include stringent cleaning and sanitation procedures, microorganisms can remain in a viable state on surfaces (Marouani-Gadri et al., 2009), or once established, remain as persistent strains within the industrial workplace (Carpentier and Cerf, 2011).

Bacterial retention has been shown to be affected by surface roughness and topography (Whitehead et al., 2011; Wickens et al., 2012; 2014). The influence of surface topography on bacterial adhesion is an important issue, and it has been suggested that surface roughness must not exceed R_a values of 0.8 μm (Flint et al., 2000). Alterations in chemical composition (Ma et al., 2008) and/or surface physicochemistry, may affect bacterial attachment and retention onto a surface (Abban et al., 2012; Skovager et al., 2012). Thus, the study of microbial retention on surfaces is important to enable informed decisions to be made regarding the design and use of materials in the food industry.

Magnetron sputtering, a physical vapour deposition process, can be used to deposit thin metal films or alloys onto chosen substrates

* Corresponding author at: School of Health Care Science, Faculty of Science and Engineering; Manchester Metropolitan University, Chester Street, UK, M15GD. Tel.: +44 161 247 1157; fax: +44 161 247 6831.
E-mail address: K.A.Whitehead@mmu.ac.uk (K.A. Whitehead).
[1] Current address: Département de Science et Analyse des Matériaux, Centre de Recherche Public Gabriel Lippmann, 41, rue du Brill, L-4422 Belvaux, Luxembourg.

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(Whitehead et al., 2004). The metals used in this study (Ti, Ag, Cu, Fe, Mo) were chosen for either their mechanical properties and/or their potential antimicrobial properties. Ti has good mechanical properties and corrosion resistance (Zaffe et al., 2003). Fe is the key component of stainless steel. Cu has been shown to inhibit growth of bacteria (Champagne and Helfritch, 2013). Ag has long been known for its antibacterial properties (Skovager et al., 2013). It is also lubricious and enables coatings to be self-lubricating (Kelly et al., 2009). Mo forms hard, stable carbides and is sometimes used in high strength steel alloys and it has also been suggested to have antimicrobial properties (Tetault et al., 2012; Zollfrank et al., 2012).

Three microorganisms where used in this study, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* since they have a potential disease burden associated with their contamination of food contact surfaces. The aim of this work was to determine the effect of surface properties of single phase, metal coatings to determine if a single surface parameter (chemistry, nano- or micro-topography, surface features or physicochemistry) had the greatest influence on bacterial retention (Table 1).

2. Methods and materials

2.1. Coating production and characterisation

In order to produce the substrata, prior to deposition, silicon substrates (10 mm × 10 mm samples) were cleaned with methanol (BDH). The pure metal coatings were deposited using a 99.9% Ag target, a 99.5% Ti target, a 99.5% Cu target, a 99.95% Mo target, and a 99.95% Fe target all of 150 mm diameter (Teer Coatings, Worcestershire, UK). Ag, Ti, Cu and Mo coatings were deposited using DC mode (Advanced Energy MDX) magnetron sputtering. An average power of 500 W was applied to the Ag, Ti, Cu and Mo targets at an operating pressure of 0.36 Pa with an Ar flow of 5 standard cubic cm per min (sccm). Being ferromagnetic, Fe can be a difficult material to deposit using a magnetron. Thus, Fe coatings were deposited by magnetron sputtering in DC mode (Advanced Energy MDX) with additional magnets placed behind the substrate to link the field lines from the magnetron. An average target power of 200 W was used at an operating pressure between 2.27 Pa and 2.93 Pa with an Argon flow of 8 sccm. Due to the different sputtering rates of each metal, the deposition time was varied between 3 min (Ag), 5 min (Cu), 15 min (Ti), 10 min (Mo) and 40 min (Fe).

Analysis measurements of coating micro-topography (S_a) were achieved using a white light profilometer (Whitehead et al., 2010) and topography (R_a) measurements were obtained using atomic force microscopy (Skovager et al., 2013). Physicochemistry of the coatings was determined as carried out by Whitehead et al. (2009). Surface tension parameters for polar and apolar liquids were used to calculate physicochemical parameters (van Oss et al., 1990; van Oss, 1995; van Oss et al., 1986) with modifications as described in Whitehead et al. (2009).

Table 1
Descriptions of surface measurements of the single phase metals (Anonymous, 2010).

Roughness parameter	Description
Roughness	The irregularities in the surface texture which are inherent in the production process but excluding waviness and errors of form.
R_a and S_a	Average absolute deviation of the roughness irregularities from the mean line over one sampling length or from the average absolute deviation of the surface respectively.
R_p	The maximum height of the profile above the mean line within the assessment length.
R_v	The maximum profile valley depth above the mean line within the assessment length.
R_z	The difference in height between the average of the five highest peaks, and the five lowest valleys along the assessment length of the profile.

2.2. Microbiology

Three potentially pathogenic food borne microorganisms were used in this work. *L. monocytogenes* EGDe was kindly provided by Prof. Lone Gram (DTU, Denmark). *E. coli* CCL410 was a kind gift from Dr. Brigitte Carpentier (AFSSA, France). This strain was recovered from a heifers faecal samples by the laboratory of Dr C. Vernozy-Rozand (Unité de Microbiologie alimentaire et prévisionnelle, Ecole vétérinaire de Lyon, France). This strain was selected since it is a non-pathogenic *E. coli* O157:H7 wild type strain that does not the carry *stx1* and *stx2* genes. *S. aureus* (NCIMB 9518) was kindly supplied by Campden BRI (UK). All stock cultures were stored at −80 °C, until needed for use and were recovered as described in Caballero et al. (2009).

Cultures were stored at 4 °C on agar for four weeks for ease of access. They were then replaced by fresh cultures taken from the freezer mix. In preparation for retention assays *E. coli* was inoculated onto brain heart infusion agar (BHIA) and incubated at 37 °C overnight. Ten millilitres of BHIB was inoculated with a single colony of *E. coli* and incubated at 37 °C overnight. One hundred microlitres of this culture was used to inoculate 100 ml BHIB, which was incubated at 37 °C for 18 h with shaking (200 rpm). Following incubation, cells were harvested at 567 ×*g* for 10 min and washed once, by re-suspension in sterile distilled water, vortexing for 30 s, and then centrifugation at 567 ×*g* for 10 min. In preparation for retention assays *L. monocytogenes* was treated as the *E. coli* except the cells were incubated in TSB at 30 °C and *S. aureus* was grown in nutrient broth at 37 °C. *L. monocytogenes* were grown 30 °C instead of 37 °C so that they would retain their peritrichous flagella, thus allowing the cells motive activity. All cells were re-suspended to an OD of 1.0 at 540 nm in sterile distilled water corresponding to 10^8 cfu/ml.

The microbial affinity to hydrocarbons (MATS) assay was followed according to an adapted method described by Bellon-Fontaine et al. (1996). Retention assays were carried out according to Whitehead and Verran (2007).

2.3. Statistics

The standard deviation of the mean is shown on the graphs using error bars. *p* values were calculated at the 95% confidence level using ANOVA and t-tests.

3. Results

3.1. Surface characterisation

The Ag coating demonstrated the greatest micro-topography, determined by the S_a value (21.4 nm), followed by the Cu (15.1 nm), Ti (12.9 nm), Fe (10.8 nm) and Mo (10.2 nm) (Table 2). Significant differences were observed between the S_a values for the Ag and Fe, Mo or Ti coatings ($p < 0.05$). Differences were noted between the surface features

Table 2
Surface topography measurements using *S* and *R* values of the polycrystalline, single phase metal coatings demonstrating that Ag was the roughest surface with the greatest topographical values, whilst Fe and Mo were the smoothest.

	Ti	Ag	Cu	Fe	Mo
S_a (nm)	12.9 ± 2.70	21.4 ± 1.75	15.1 ± 4.15	10.8 ± 2.73	10.2 ± 1.82
R_a (nm)	7.51 ± 0.59	6.96 ± 0.53	4.39 ± 0.75	1.46 ± 0.27	1.45 ± 0.17
R_p (nm)	50.4 ± 16.2	48.1 ± 12.6	49.4 ± 19.6	14.6 ± 5.73	9.86 ± 2.00
R_v (nm)	15.90 ± 6.05	38.6 ± 4.55	33.9 ± 7.14	15.2 ± 6.98	11.2 ± 1.29
R_z (nm)	7.51 ± 0.59	9.09 ± 0.98	6.09 ± 1.01	1.85 ± 0.38	1.87 ± 0.18