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

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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 was 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 trends 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.

Keywords: Food; Metals; Surface topography; Bacterial retention; Physicochemistry; Chemistry

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., 2010), 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 (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 et al., 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.

2. Methods and Materials

2.1. Coating production and characterisation

In order to produce the substrata, prior to deposition, silicon substrates (10 mm x 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) was 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).

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 ºC, 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 sec, and then centrifugation at 567 × *g* for 10 min. In preparation for retention assays *L. monocytogenes* was treated as the *E. coli* except cells were incubated in TSB at 30 ºC and *S. aureus* was grown in nutrient broth at 37ºC. *L. monocytogenes* were grown 30 ºC instead of 37 ºC 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 of the individual metals. The Ti coating had randomly spaced, sharp surface protrusions (100 nm – 300 nm wide, 33.2 nm ± 11.1 nm height) (Figure 1a and 2a), whereas the Ag coating demonstrated larger (100 nm – 750 nm wide, 43.4 nm ± 8.58 nm height), closely packed, randomly distributed rounded surface structures (Figure 1b and 2b). The Cu coating had a dense, nano textured form with some linear features apparent (50 nm – 300 nm wide, 41.7 nm ± 13.4 nm height) (Figure 1c and 2c). The Fe coating had sharp, periodic protrusions (50 nm – 200 nm wide, 14.9 nm ± 6.36 nm height) (Figure 1d and 2d). The Mo coating demonstrated randomly spaced, densly packed, linear shaped round surface protrusions (100 nm – 400 nm wide, 10.5 nm ± 4.65 nm height) (Figure 1e and 2e). The height of the coating features for all the coatings was in the nano range.

In terms of nano topographies (*R* values), generally, Ti demonstrated the highest R values followed by Ag, Cu Fe and Mo (Table 2). There was a significant difference between the *R* values for the Ag, Fe and Mo surfaces, with the exception that there was no significant difference observed for the R_v value between the Fe and Mo surfaces.

The Mo coating was found to be the most hydrophobic surface (-48.90 mJ/m^2) (Figure 3). The Ti, Ag and Cu coatings were similar and partially hydrophilic (range -13.03 mJ/m^2 to – 11.72 mJ/m^2), whilst the Ag coating was hydrophilic (-0.08 mJ/m^2). For the apolar component, Mo demonstrated the most apolar surface (36.87 mJ/m^2), with Cu demonstrating the least apolar surface (8.38 mJ/m^2). For the polar, electron accepting γ_s^+ and electron donating γ_s^- surface components the Ag coating demonstrated the highest values whilst the Mo coating demonstrated the lowest.

3.2 Microbiology

L. monocytogenes was demonstrated to have a strongly hydrophobic cell surface, with *S. aureus* and *E. coli* being strongly hydrophilic; *E. coli* slightly more than *S. aureus*.

The different bacteria were not retained in the same percentage coverage to the same coatings. *L. monocytogenes* demonstrated the greatest percentage coverage on the Ag coating (4.27 x 10^4 cells/cm^2) and the lowest on the Fe coating (1.74 x 10^4 cells/cm$^{2)}$ (Figure 5a). *E. coli* retained the greatest percentage coverage on the Mo coating (1.65 x 10^4 cells/cm^2) and the least on the Fe coating (3.39 x 10^2 cells/cm^2) (Figure 5b). *S. aureus* was retained in the greatest percentage coverage on the Fe metal coating (1.48 x 10^4 cells/cm^2 (Figure 5c) and the least on the Ag coating (6.35 x 10^3 cells/cm^2). There was a significant difference for the *L.*

monocytogenes cells/cm^2 retained between the Ag and Fe coatings, for the *E. coli* between the Mo and Fe coatings and for the *S. aureus* between the Fe and Ag coatings.

4. Discussion

The presence of potential food borne pathogens in the food processing industry is of increasing concern. It is known that surface properties such as physicochemistry (Skovanger et al., 2013), chemistry (Ma et al., 2008; Whitehead and Verran 2007) and/or topography (Palmer et al., 2007; Whitehead et al., 2005) may affect cell retention.

The materials deposited were of different chemistries, and presented differently shaped topographies. Surface chemistry was found to affect the numbers of bacteria retained, with the greatest differences being observed on the Mo, Ag and Fe surfaces. It was found that there was no trend in the shape of the surface features and bacterial retention. Although all the coating topographies were well below the R_a values of 0.8 μm previously determined to be hygienic for the food industry (Flint et al., 2000) it was demonstrated that there was a significant difference on the effect of surface micro-topography but not nano-topography on bacterial retention. Results from the *L. monocytogenes* retention assays suggest that these bacteria were most influenced by surface roughness since they were retained in the greatest numbers on the rough hydrophilic surfaces and least on the smooth hydrophilic surfaces. *E. coli* was most influenced by the physicochemical status of the surface since it was retained in greatest numbers on the smooth hydrophobic Mo, with the lowest γ_{AB}, γ^+ and γ^- values and lowest on the partially hydrophilic, smooth Fe coating. *S. aureus* was retained in the greatest numbers on the smooth, partially hydrophilic Fe coating and in the lowest numbers on the roughest, hydrophilic Ag coating which suggests that surface topography had the greatest influence on *S. aureus* retention to surfaces. It is important to note that the viability of the attached cells was unknown.

There was no trend found between the hydrophobicity of the cells and retention and the physicochemistry of the surfaces. It might be that the MATS assay may be better used to determine trends between bacterial attachment and adhesion to substrata, since cell physicochemical factors are prevalent in initial cell attachment to a surface, rather than in the effects of cell retention.

The Cu and Ti coating displayed similar mid-range results for their topographies and physicochemistries, and thus did not have a significant effect on microbial retention when compared to the other coatings.

Conclusion

The results demonstrated that the different coatings exhibited a range of nano-topographies and physicochemistries. These results suggest that the effect of surface properties on cell retention is genus specific. It also highlights that different aspects and measurements used to describe the surface properties may be linked to factors affecting microbial retention hence, the use of surface roughness or physicochemistry alone is no longer adequate.

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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

Table 2 Surface topography measurements using *S* and *R* values of the polycrystalline, single phase metal coatings demonstraing 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

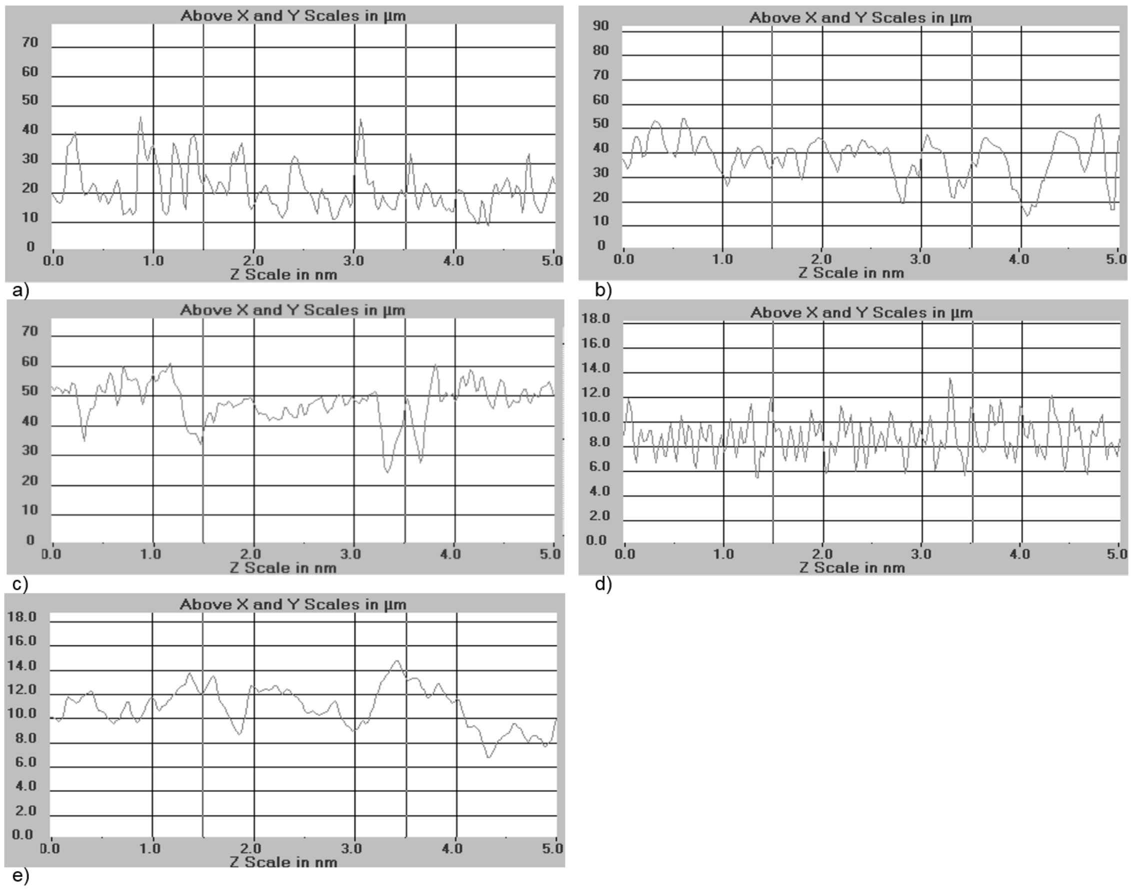

Fig. 1. AFM two-dimensional profiles of surface topographies of the single phase metals a) Ti b) Ag c) Cu d) Fe and e) Mo demonstrating the differences in the shape and scale of the surface features across one X axis. Z heights are not on the same scale in order to allow visualisation of surface features

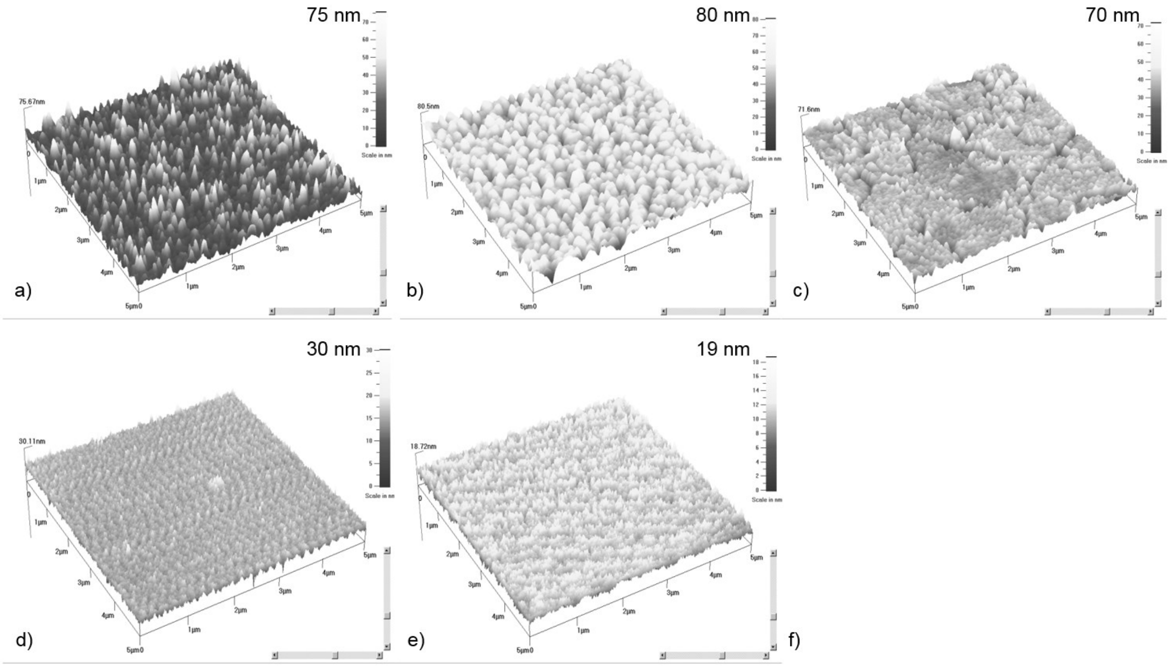

Fig. 2. AFM images of surface nano-topographies of the single phase metals a) Ti b) Ag c) Cu d) Fe and e) Mo demonstrating the surface features. Z heights are not on the same scale in order to allow visualisation of surface features

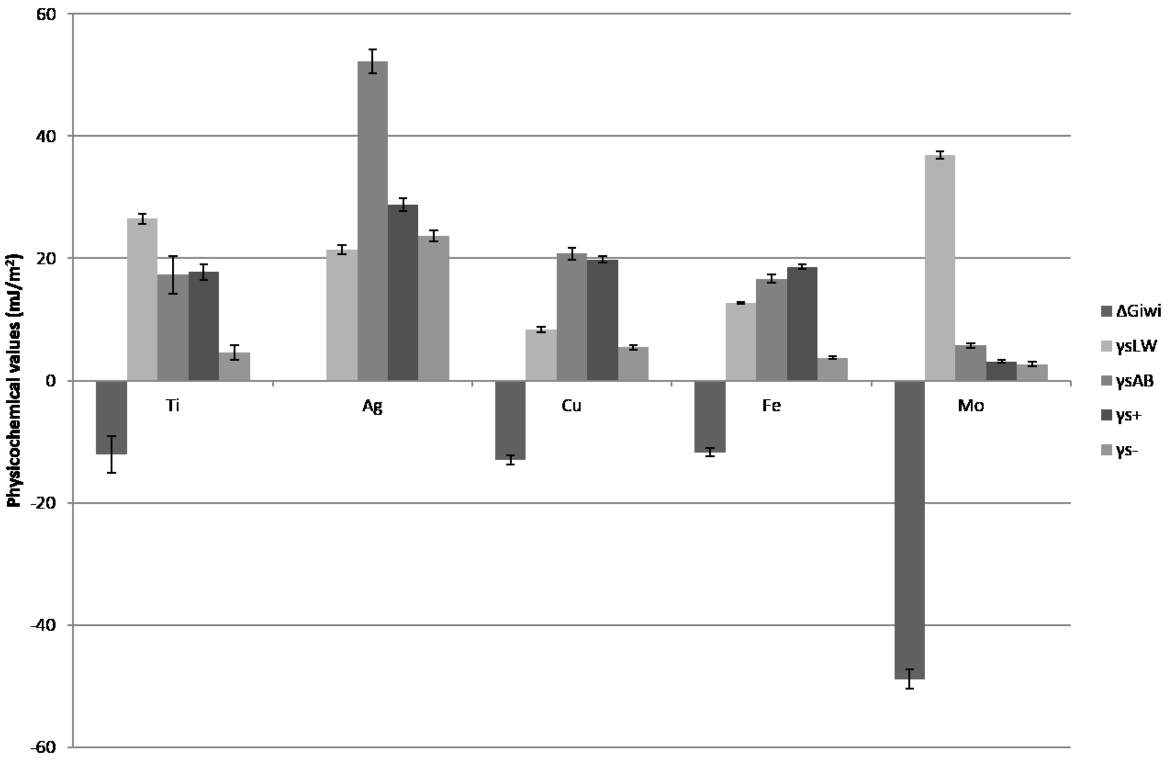

Fig. 3. Physicochemical measurements of single phase metals surfaces demonstrating that the surfaces had a range of surface properties with silver being the least hydrophobic and molybdenum the most hydrophobic

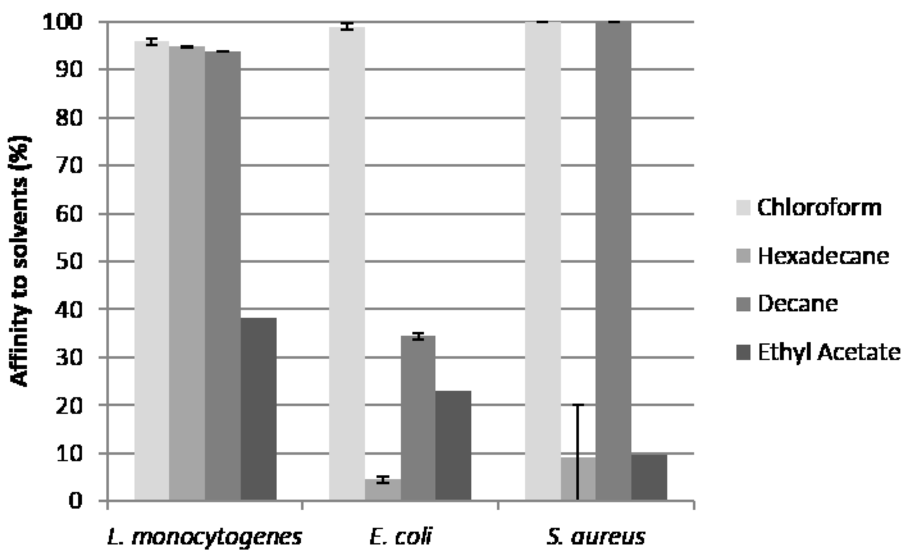

Fig. 4. Cell affinity to solvents following the MATS assay demonstrating that each bacterial species demonstrates unique cell surface properties. As demonstrated by % cell affinity to hexadecane, *L. monocytogenes* was the most hydrophobic, whilst *E. coli* was the least

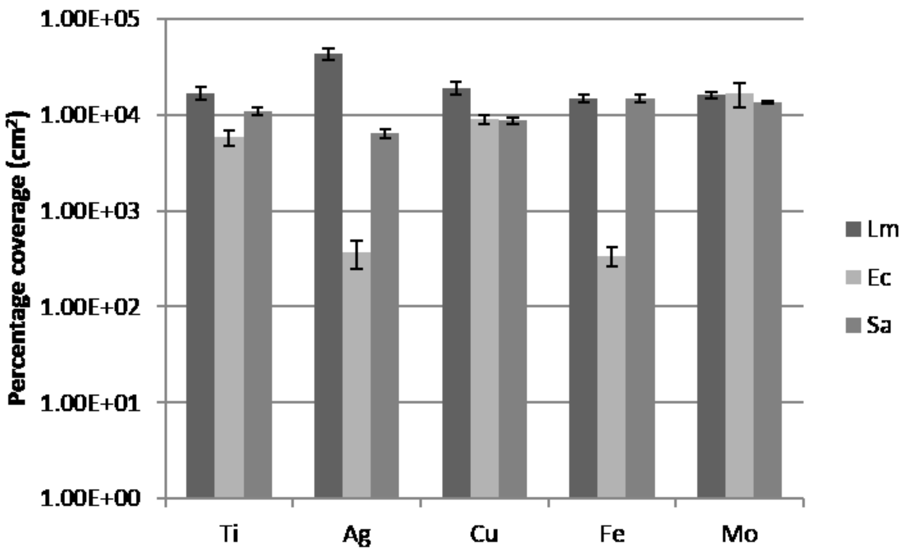

Fig. 5. Number cells retained on the surface following retention assays a) *Lm* = *L. monocytogenes* b) *Ec* = *E. coli* and c) *Sa* = *S. aureus*