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Modification of Stainless Steel with Zinc and Niobium Oxides for Antimicrobial Effect

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The microbial adhesion of pathogens on surfaces, followed by the formation of biofilms, constitute one important causes of diseases transmitted by foods. Biofilm control in the food industry is critical since biofilm removal is challenging. Thus, the functionalization of surfaces has been a strategy to prevent the multiplication of bacteria. This study aimed to functionalize stainless steel surfaces with zinc and niobium oxides and to analyze its antimicrobial capacity of Escherichia coli. In addition, the roughness surface was also investigated. The free energy of hydrophobic interaction was calculated by measuring the contact angle. The results showed that surface functionalization with metallic oxides efficiently controlled E. coli adhesion, achieving more than two decimal reductions in the initial population. It was found that the deposition of oxides modified the hydrophobicity of the stainless steel surface, making it hydrophilic, which may have added to the effect of functionalization for the antimicrobial efficiency of the obtained surface. The surfaces functionalized with zinc and niobium oxides had the highest roughness. Thus, surfaces with Nb and Zn oxides can be a promising alternative for application in the food industry to help control adhesion and obtain the final product of microbiological quality.

Introduction

As the global population grows, the demand and consumption of food are steadily increasing worldwide [**1**]. This has led to one of the most prominent contemporary challenges globally, which is ensuring the availability of sufficient safe and nutritious food for a growing population [**2**]. Unfortunately, the reduction in the supply of adequate and safe food has contributed to an increase in obesity rates, deficiencies in essential micronutrients, and the prevalence of diseases related to food restriction [**3**].

The World Health Organization (WHO) estimates that the consumption of contaminated food is responsible for approximately 600 million cases of foodborne disease transmission each year, resulting in 420,000 deaths [**4**]. Similarly, the United States Centers for Disease Control and Prevention (CDC) reports that around 48 million people contract foodborne illnesses annually, with 128,000 requiring hospitalization and 3,000 resulting in death [**5**].

Beyond the toll on human health, consuming food contaminated with pathogens also has significant economic impacts. These impacts can devastate not only consumers but also nations, food traders, and food companies. Pathogenic bacteria, viruses, and protozoa can infiltrate food at various stages of its procurement, including primary production, harvesting, transportation, processing, storage, distribution, and preparation [**6**].

Among the various contamination points, food contact surfaces play a particularly critical role. Surfaces that come into contact with food and the water used in their cleaning process can become contaminated by pathogens. These surfaces have been identified as a significant factor contributing to foodborne illnesses [**1**]. In the food industry, the practical application of cleaning and sanitizing methods, with an emphasis on pathogen removal, is considered crucial for ensuring food safety.

In this sense, chlorine-based sanitizers are widely used in the food industry for this purpose. However, it is worth noting that free chlorine can react with natural organic matter and transform into inorganic chloramines, which not only reduce antimicrobial activity against biofilms but also have carcinogenic properties [**7**]. Moreover, the environmental implications cannot be ignored, as chlorine-based compounds in surface cleaning effluents can disrupt ecosystems, alter oxygenation levels, and pollute aquatic environments [**8**].

Given these concerns, there is a growing trend in research to explore viable alternatives to chlorine use in the food industry. One promising strategy is the modification of stainless steel surfaces, commonly used in food industries, with metallic oxides to reduce their adhesion properties [**8**]. This approach not only has the potential to minimize the need for chlorine in the sanitization stage but also contributes to food safety by inhibiting the formation of pathogen biofilms on surfaces and reducing the risk of food contamination.

Additionally, the application of metal oxides as antimicrobial agents presents a solution to the global challenge of antimicrobial resistance to conventional antibiotics. Thus, metal-based biocides are among the most popular for a wide range of applications, including industrial, agricultural, marine, residential, and medical, and can be deposited or adsorbed onto a substrate [**9**]. Their attractive characteristics include durability and high stability with low toxicity to mammalian cells compared to organic equivalents [**10**].

In this context, the objective of this study was to achieve stainless steel surfaces with varying degrees of polishing, referred to as polished and sanded surfaces. Additionally, the study aimed to modify only the sanded surfaces using zinc and niobium oxides separately, with the goal of observing a reduction in microbial load. Furthermore, the study also intends to assess whether the inclusion of the metal oxide modification step can eliminate the need for surface polishing during the manufacturing process.

Experimental

Sample preparations

In the stainless steel surface study, AISI 304 stainless steel (donated by Arcelor Mital Industry) samples with dimensions of 50 x 90 mm and thickness of 5 mm were used. The samples consist of polished-only coupons and sanded-only coupons for the tests herein performed in order to investigate the influence of the surface roughness over the bacteria adhesion.

The polishing and sanding processes were performed manually and unidirectionally, using a manual grinder (MKS 16), an orbital sander (Bosh Gss Ae 190W), and a radial polisher (DeWalt) for polishing. The tool used to perform the sanding operations is angle grinder tool that is simply a method for rubbing abrasive particles against the surface of a workpiece to create a random, non-linear surface texture. Sanding operations are important techniques of the manufacturing process and are made up of many tiny abrasive grains held by abrasive bonding material.

After the above mentioned preparation, the samples were cleaned with neutral detergent, rinsed with distilled water, dried, and sanitized with alcohol 70% (v/v). After sanitizing, they were rinsed again with distilled water, dried at 60°C, and autoclaved at 121°C for 15 minutes.

Functionalization of stainless steel coupons with zinc and niobium oxides

Zinc oxide and niobium oxide films were deposited on sanded stainless steel coupons by reactive sputtering technique. To obtain high-quality films, a 2 in. diameter, 99.9% purity Zn target purchased from Kurt Lesker was used. The Niobium target 99.99% pure was donated by CBMM Brasil Co. The residual pressure in a chamber was 0.006 mTorr sustained by a turbomolecular pump. The deposition was performed using Argon (White Martins 99.99%) and oxygen (White Martins 99.99%). The gases admittance and control were performed by needle valves (Edwards Co.) using 4.0 and 1.0 mTorr for Argon and Oxygen, respectively for Nb and Zn oxide. After functionalization, the coupons were forwarded for microbiological analysis according to the methodology presented in section 2.5. Besides microbial analysis, the coupons were also submitted to contact angle and roughness measurements, according to description below.

Hydrophobicity analysis

The contact angles of the different surfaces were determined and measured using the sessile drop method with goniometer equipment (Kruss Advance for Drop Shape Analyzers Version 1.7, Hamburg, Germany). The objective of this analysis is to evaluate the influence of the roughness, as well as of the metallic oxides films on the hydrophobicity of the surfaces. Three liquids with different polarities were used (distilled water, formamide (PA), and α-bromonaphthalene (PA)) to obtain the surface energy. Nine measurements were performed in different points for each liquid and sample, as described by Van Oss [**11**].

Surface roughness analysis

The surface roughness was measured with a portable roughness meter (Surtronic 3+ model 112/1590) manufactured by Taylor Hobson. The instrument has a diamond probe needle with a tip radius of 5 µm, resolution of 0.01 µm, operating with a load range of 150 to 300 mg.

Study of microbial adhesion

After sanitizing the surfaces, the polished and sanded surfaces modified with metallic oxides were subjected to the microbial adhesion study using *Escherichia coli* ATCC 25927 (INCQS/FIOCRUZ – Brazil) (approximately 5 Log $CFU \cdot mL^{-1}$), previously grown in BHI broth (Prolab, Brazil), in aerobic conditions for 24 hours at 37ºC.

The incubation was carried out under the static condition to obtain homogeneity in the fouling levels on the surface of the geometries and to isolate the effect of hydrodynamics during adhesion and biofilm formation [**12**].

The surfaces were removed and washed with peptone water (0.1%) to remove non-adhered cells. Next, the swab method was applied to remove sessile cells. Then, the swab was transported to a tube containing 0.1% peptone water and submitted to vortex rotation for cell removal and subsequent counting in Petri dishes containing Plate Count Agar (PCA) medium for analysis of the cells adhered to the surfaces. The entire procedure was performed in duplicate. The plates were incubated for 24 hours at 37°C. The counting result was expressed in $CFU \cdot cm^{-2}$.

Experimental design

The experiment was completely randomized. The bacteria count on the coupons were expressed as CFU $Log.cm^{-2}$. Data were analyzed by Analysis of Variance (ANOVA) and the means were compared using the Tukey's test at a 5% probability, using the statistical software STATISTICA, version 7.0. The experiment was carried out in three replicates.

Results and discussion

From the results of the water contact angle values (**Table 1**), we can be seen those surfaces polished and sanded were considered the most hydrophobic. This analysis is a qualitative methodology. However, there are differences in the literature regarding the classification of the surface as hydrophobic or hydrophilic, depending on the value of the angle formed with water. For Choi [**13**] the surface is hydrophobic when the contact angle is greater than 70°, while the hydrophilic surface has a contact
angle less than 70°. For Van Oss and Giese [**14**], angles less than 50° indicate a hydrophilic surface and angles greater than 50° a hydrophobic one. For Vogler [**15**], a hydrophobic surface should have a water contact angle greater than 65°. Considering all these classifications, the surfaces with zinc and niobium deposition are hydrophilic when only water was assessed to determine the contact angle.

Table 1 also presents the hydrophobic interaction free energy (ΔG_{sws}^{TOT}). The ΔG^{TOT} calculated parameter expresses the variation of interfacial interaction free energy between the molecules of the material immersed in water and is a quantitative criterion that it is the most appropriate measure of hydrophobicity. This quantitative criterion defines the hydrophobicity of a surface efficiently because it considers van der Waals forces, electrostatic interactions, and polar interaction forces, which may be repulsive or attractive.

We observed that the surfaces only sanded was classified as the most hydrophobic surface, and the least hydrophobic surface was the zinc-modified surface, presenting ΔG_{sws}^{TOT} of 0.26 mJ m^{-2}. All surfaces differed statistically ($p < 0.05$). This shows that the sputtering of the oxides under the stainless steel surfaces could cause a change in the hydrophobicity values of the stainless steel surfaces. Thereby, the presence of the metallic oxides may have contributed to the decrease in hydrophobicity on the surfaces of modified steel.

Thus, it is important to mention attachment surface properties, such as, electrostatic charge, hydrophobicity, interface roughness, and topographic impact, affect the overall hygiene status of the surface and therefore biofilm formation [**16**].

Table 1. Average values of the water contact angle and components of the interaction free energy (ΔG_{sas}^{TOT})

Sample	Contact angle (°) with the water	(ΔG_{sws}^{TOT}) mJ m^{-2}
Surface polished	63.52 ± 2.80	-13.67[a]
Surface sanded	75.54 ± 5,72	-66.60[b]
Zinc oxide	47.19 ± 6.92	0.26[c]
Niobium oxide	39.41 ± 2.55	10.96[d]

[a,b,c,d:] Equal letters in the same column indicate statistically equal means by Tukey's test at 5% significance.

(ΔG_{sws}^{TOT}): free energy of hydrophobic interaction between surface molecules and water.

In addition, the characteristics of the microorganism, such as cell hydrophobicity, electrical charge, production of extracellular substances, cell appendages and environmental factors such as pH, temperature, and nutrients influence cell adhesion to the surface [**17**].

Table 2 shows the average roughness values of the surfaces tested. By the analysis of variance (ANOVA) the treatments were significant on surface roughness. Thus, we observed that the polished surface presented the lowest roughness, followed by the sanded surface. The surfaces treated with oxides showed the highest roughness compared with polished and sanded samples. The niobium oxide resulted in a higher roughness compared with zinc oxide surface.

Table 2. Average Roughness values -Ra (µm) of the studied stainless steel surfaces and $CFU.cm^{-2}$ values of *Escherichia coli* adhered on different 304 stainless steel surfaces.

Samples	Ra	Log
Surface polished	0.03 [a]	7.22 [a]
Surface sanded	0.26 [b]	7.12 [a]
Zinc oxide	0.77 [c]	5.47[b]
Niobium oxide	0.91 [d]	5.38[b]

[a,b,c,d:] Equal letters in the same column indicate statistically equal means by Tukey's test at 5% significance.

In general, higher surface roughness increases the surface area available for bacterial attachment and provides a framework for adhesion [**18**]. Although surface roughness is often positively correlated with the degree of bacterial adhesion and biofilm formation, it has been noted in the literature that higher surface roughness, in some cases, can also result in reduced bacterial adhesion [**19,10,20**]. Studies have reported a lack of correlation between surface roughness and the number of adhered bacteria [**21,22**].

In this study, the polished and sanded surface treatment presented statistically the same degree of contamination, with no significant difference between the surface finishes. On the other hand, the coated surfaces by Nb and Zn oxides were able to produce a reduction of approximately 2 log $CFU.cm^{-2}$ in cell adhesion on the surface (**Table 2**). In this sense, it was found that there was no correlation between the level of adhesion and roughness, as different roughness (polished and sanded surface) showed the same levels of cell adhesion. Likewise, the surfaces treated with the oxides showed different degrees of roughness and still showed the same level of microbial contamination on the surface.

In this context of comparison of bacterial adhesion between polished and sanded surfaces, Schlisselberg and Sima Yaron bring an interesting approach. They found in their study that electropolishing surface was the least colonized surface 1 h after initial attachment. Regarding biofilm formation for a long period of time, the Mechanical Sanded surface seems to be the least susceptible surface with the least amount of biofilm formed [**23**].

According to the results presented, we can state that the change in the hydrophobicity value may be a good indication, in this study, of modification on the surface from the deposition of the oxide, making it less adhesive. Gomes [**24**] also revealed in their work that bacterial adhesion was lower on hydrophilic surfaces, where they observed that hydrophobic materials (water contact angles of 115.4 ± 0.4, 67.0 ± 1.7, and 79.3 ± 0.9) showed more significant bacterial colonization than hydrophilic material (water contact angle of 47.0 ± 0.4).

On the other hand, Oh [**25**] observed that the extent of adhesion of *E. coli* and *S. aureus* was greater on hydrophilic substrates (water contact angles 54.5 ± 1° and 59.3 ± 0.7°) than on hydrophobic substrates (water contact angles 92.5 ± 0.7, 98.6 ± 0.8 and 108.4 ± 0.7). Pranzetti [**26**] reported, for *Marinobacter hydrocarbonclasticus* and *Cobetia marina*, that bacterial adhesion was higher on 11-aminoundecanethiol hydrochloride surfaces, which showed a water contact angle of 60 ± 2° (i.e., hydrophilic) than on 1-hexadecanethiol surfaces, which showed a contact angle of 105 ± 4° (i.e., hydrophobic. Increased hydrophobicity can also lead to decreased adhesion, as in the case of the study by Torqueti [**8**] in which the stainless steel surface functionalized with silver had its hydrophobicity increased relative to the untreated surface and adhesion by *E. coli* and *S.aureus* reduced. It is also highlighted that hydrophobic materials often inhibit the adhesion of bacteria due to their specific self-cleaning property [**27**] and that some hydrophilic or superhydrophobic materials also exhibit anti-adhesion effect due to their strong electrostatically induced hydration layers that build a steric barrier to adhesion [**28**]. However, the fact that there are no precise results on adhesion and hydrophobicity may lie in the various methods and bacterial strains employed. The overall interaction is probably being established for various reasons [**29**].

In this study, the decimal reduction may have been a synergistic effect between the change in surface hydrophobicity and the antimicrobial activity of the oxides that promoted microbial inactivation when microbial cells were deposited on them.

Unlike the use of traditional antimicrobial agents, the characteristics of these oxide functionalized surfaces are unlikely to contribute to the formation of antimicrobial-resistant bacterial strains. This is because bacterial inactivation mechanisms do not involve attacking specific targets within the bacterial cell [**30**]. Furthermore, contact killing, in theory, can work for a relatively long time, assuming that the surface can be reactivated after the dead microorganisms are removed [**31**].

Dwivedi [**32**] suggests that the mechanism of antibacterial activity of ZnO-NPs is based on their ability to induce oxidative stress. The released Zn^{+} ions interact with the thiol group of bacterial respiratory enzymes, increasing the production of reactive oxygen species (ROS) and causing oxidative stress in the bacterial cell. This oxidative stress damages bacterial membranes, DNA, and mitochondria, resulting in the bacterium's death [**33,34**].

Recent studies have demonstrated that ZnO NPs effectively combat multidrug-resistant bacteria (Tiwari et al., 2018) and prevent biofilm formation [**35**]. Others have studied the application of ZnO in films coating various surfaces and its antimicrobial effect. As in work by Mizielińska [**36**], in which PLA (polylactic acid) films with ZnO nanoparticles incorporated obtained a reduction of 1.7 log cycles in the number of *S. aureus* and also Fontecha-Umaña [**37**] in which polyester surfaces coated with ZnO had reductions of up to 1.19 log cycles for *S. aureus* and 2.07 for *E. coli*.

An antimicrobial polyvinyl chloride (PVC) surface also was obtained by covalent binding of zinc oxide nanoparticles. These functionalized surfaces showed excellent antibacterial and antifungal activity and were also able to prevent biofilm formation. Microbiological investigation indicated that the antimicrobial power of ZnO-modified PVC films was most likely due to a combination of many factors, such ROS production and Zn^{+2} release [**38**].

From the exposed results, it can be stated that the procedure consisted by zinc and niobium oxides deposition by sputtering is promising to be used on food processing surfaces by promoting the reduction of bacterial adhesion since the surfaces modified with oxides were the ones that showed lower values of adhered cells compared to all other surfaces with different polishes and untreated. Moreover, it is also shown as an economically favorable alternative for food industries since the high cost of polishing the stainless steel surface is known.

Therefore, it is essential to mention that although Brazil has the largest reserves of Nb, similar studies using niobium are scarce in the literature, with a few works published in the field of medical implant [**39,40**] but its excellent potential is visible. See a recently published review regarding the progress in coatings containing niobium oxide for biomedical applications, where they highlight that niobium oxide films applied to biomaterials can overcome deficiencies in these supports, such as resistance to corrosion, and also present new properties such as antibacterial activity [**41**].

Future perspectives also are based on testing a combination of metal oxides on the stainless steel surface to evaluate further antimicrobial efficiency, and to study the durability of the coating after exposure to chemical agents, such as those used in the food industry in the CIP (cleaning-in-place) cleaning process.

Conclusion

The surfaces evaluated showed differences in hydrophobicity and roughness values and the number of adhered cells. In this study, the surface characteristic influenced the number of adhered *E. coli* cells. The results showed that the modification of the surface with metallic oxides presented more efficience than polishing in the microbial control. In addition, the food industry must be concerned with surface characteristic since it needs to offer consumers products of microbiological quality that do not offer health risks, furthermore the modification of surfaces has been a strategy for coating to prevent the fixation and/or multiplication of bacteria.

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Conflicts of interest

I declare that there is no conflict of interest on the part of the authors.

Keywords: *Escherichia coli*; biofilm; stainless steel; zinc oxide; niobium oxide.

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Authors Biography

Professor Rogerio is an expert in the use of cold and reactive plasma-based techniques for thin film deposition as well surface funcionalization. Professors Emiliane and Leticia are experts in the area of microbial control on food processing surfaces. Professor Vitor has expertise in machining and surface fabrication. The participating students (Bruna, Leonardo and Lucas) are of excellence compared to their peers.

Graphical Abstract

Surfaces modified with metal oxides such as zinc and niobium are able to change characteristics such as hydrophobicity and roughness, and reduce microbial adhesion on stainless steel. Therefore, they have the potential to reduce the use of inorganic chlorine compounds, commonly used as sanitizers in the food industry.