European Food Research and Technology (2024) 250:203–211
https://doi.org/10.1007/s00217-023-04378-8

ORIGINAL PAPER

Effectiveness of nanoscale silicon dioxide-coated picker fingers on *Salmonella* Enteritidis and *Escherichia coli*

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Received: 30 June 2023 / Revised: 10 September 2023 / Accepted: 16 September 2023 / Published online: 5 October 2023

Abstract

In poultry slaughtering, cross-contamination with *Salmonella* Enteritidis is a constant ongoing challenge. Interaction between food contact surfaces can potentially transfer pathogenic material like feces from carcasses to another one. One approach to break this chain is to modify surfaces that frequently come into contact with the animal during the slaughtering process. Surface alterations like nanoscale coatings have already been successfully applied in various fields to lower the bacterial load. The aim of the study was to compare bacterial attachment, proliferation and detachment of *Salmonella* Enteritidis and *Escherichia coli* on uncoated and on nanoscale silica coated rubber picker fingers at laboratory scale. It was shown that both target organisms did not adhere less to coated surface than to uncoated picker fingers, whereas no difference in bacterial growth or detachment was detected. It can be concluded that the coating used in this study did not contribute to a reduction of the bacterial load on this surface in the specific experimental setups employed. Further studies should focus on whether nanoscale surface modifications achieve improved results under more practical conditions and whether other factors such as surface durability can be influenced by a coating.

Keywords Attachment · Surface modification · Bacterial load · Poultry · Food contact surfaces · Cross-contamination

Introduction

Salmonella (S.) enterica cause the second most common bacterial enteritidis in humans with approximately 60,000 cases in 2021 in Europe [1]. Due to the past control programs in Europe, the number of infections caused by *S. enterica* in primary production of poultry and pigs could be reduced by accompanying steps such as logistical slaughtering. This have an effect on cross-contamination by decreasing the bacterial load at the various stations of the process, but it appears that these measures are not yet sufficient [2]. Even carcasses with a negative *Salmonella* status can be contaminated with *Salmonella* during slaughter due to soiled equipment [3]. Despite this, cases of salmonellosis are still on a high level, most recently with about 9000 cases in 2022, in Germany [4]. *Salmonella* Infantis is the most common serovar recovered in the slaughtering process environment, and in terms of zoonotic potential, serovars Typhimurium and Enteritidis appear to be the most clinically important ones [5, 6].

One of the main causes for salmonellosis is still the consumption of improper heat treatment contaminated food, besides of eggs or egg products, in particular pork and poultry meat [7]. Slaughtering process risk such as carcass contamination and cross-contamination are in the focus [8], where potentially pathogenic organic material is passed from carcass to carcass via equipment such as conveyer belts, shackles or other food contact surfaces [9, 10]. This occurs particularly during poultry defeathering, as large amounts of organic material accumulate in this highly mechanized process, and the exchange rate between carcasses is correspondingly high [11]. Pacholewicz et al. [12] showed that the defeathering step should still be given special attention with respect to microbial cross-contamination. In a study, in which swabs and water samples were collected from the slaughter line before slaughter, it was found that the area of plucking had the highest *Salmonella* prevalence of 10.4% (for the defeathering machine: 17.0%) [6]. Additionally, in the subsequent highly mechanized evisceration, feces can leak from the organs due to the removal of the intestinal

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convulsions and contaminate subsequent carcasses or the machines themselves [13, 14].

It is already known that different *Salmonella* serovars such as Sofia, Typhimurium, Virchow and Infantis have the ability to adhere to different substrates commonly used in the slaughtering process, for example rubber and stainless steel [15]. Most serovars are also able to form biofilms on stainless steel depending on prevalent conditions [16]. Therefore, it is of interest to influence the interaction between surface and carcass in a way that adapting the surface and makes it unfavorable to bacteria. In the slaughtering process itself, various materials with an increased risk of cross-contamination have already been identified, such as generally stainless-steel surfaces, conveyor belts, picker fingers [10] and cutting boards [17]

On natural surfaces like plants or insects, topographical structures in nanometer range could be detected, which protect the corresponding living being against environmental influences [18, 19]. Modification of contact surfaces by direct material changes or coatings has long been known in other fields such as in human medicine like implants in dentistry [20] or active packaging with nanocoating in food industry [21, 22]. There are various considerations about the exact functional principle of these modified surfaces. Many factors play a role in the bacteria-surface interaction under consideration: first, the surface itself with its properties such as chemical composition, surface tension, surface free energy, as well as topographic changes such as roughness and the associated wettability [23, 24]. The bacterium or bacteria [18] and the environment affecting the interaction must also be taken into account [24], which is why the effect of a coating can vary in each case. The surface modifications change the wettability of the surface to hydrophobic, so that the self-cleaning effect of the coating can occur [25, 26]. Other studies report a rupturing of the bacterial cell wall and thus a bactericidal effect of such surface modifications [27, 28]. The exact principle of such complex surface modifications and the interaction of the different parameters of surface, bacterium and environment have not been completely understood and explained until today [23].

In past studies, different materials were directly modified or coated. Nanostructured gold surfaces, for example, were shown to be bactericidal against *Staphylococcus* (*St.*) *aureus* showing cell deformation and cell rupture after two hours of incubation [29]. The textured surface of other metals such as aluminum allows *Escherichia (E.) coli* and *Listeria (L.) innocua* to attach better when they have a nanoporous topography compared to nanosmooth surfaces [30]. It was also discovered that different nanometer scale topographies of TiO_2 grown on titanium, commonly used in human medicine, affected the attachment of bacteria such as *St. epidermidis* by killing 99% of bacteria during two hours of contact time with nanostructured surface [27]. PMMA (poly(methyl methacrylate)) as an example of plastics or synthetic materials is capable of forming nanopillar structures, which was found to result in higher dead fraction of adherent *E. coli* (16–141%) and thus reduced viability compared to flat controls [31]. However, also coating of surfaces seems promising, which is realized in some cases by the sol–gel technology, which allows to coat the surface by dipping or spraying [32]. Furthermore, coating of diamond-like carbon nanocomposites with embedded silver nanoparticles was the most effective in terms of reducing *L. monocytogenes* and *Campylobacter jejuni* compared to uncoated silicon, stainless steel and HDPE (high density polyethylene) [33]. Titanium discs coated with a thin tantalum film led to reduction in adhesion and growth of *Streptococcus mutans* and *Porphyromonas gingivalis* [20].

The application of nanoscale coating on surfaces exposed in the scalding room and defeathering area like picker fingers could influence the bacterial behavior and decreases the prevalence of *Salmonella* [6]. It has already been shown that nanoscale silica coating of stainless steel tends to reduce the attachment in *Salmonella* Enteritidis and *E. coli*, and with some tendency to increase detachment of *E. coli* from coated stainless steel [34]. In addition, a study revealed decreased adhesion for *Pseudomonas aeruginosa* (57%) and *St. aureus* (20%) on nanoscale gold coated polystyrene surfaces compared to microscale surfaces [35]. Ivanova et al. [36] showed that synthesized black silicon as a surface material exhibits fine needle-like structures, which is bactericidal to Gram-negative as well as Gram-positive bacteria. In another study, it was shown that the concentration of 4% of a coating with silica-titania nano core-shells showed the best antibacterial effect against *E. coli* and *Bacillus* [37]. Di Cerbo et al. [38] showed that nanotechnological coating of variously rough stainless steel may be due to the synergistic effect of reduced bacterial adhesion and increased hydrophobicity.

Therefore, the aim of this study is to investigate the bacterial attachment, growth and detachment of *Salmonella* Enteritidis and *E. coli* on nanoscale silica coated and uncoated picker fingers. *E. coli* is chosen as an indicator bacterium for fecal contamination of surfaces. The presence of the pathogen suggests a possible contamination of surfaces with *Salmonella* spp. [39]. When applied as a marker organism, *E. coli* shows a similar distribution pattern on poultry in defeathering machines as naturally occurring microorganisms on carcasses [40].

Materials and methods

Bacterial material

The bacterial strains employed, *Salmonella* isolate 19-SA00115 (*S.* E.) and *E. coli* isolate (20-AB00467) were

obtained from the German Federal Institute for Risk Assessment and were both isolated from chicken meat samples. The cryopreserved isolates (− 80 °C, Cryobank, Mast Group Ltd., Germany) were grown on tryptone glucose yeast extract agar (Plate Count Agar; PC; TN1189; sifin diagnostics GmbH, Germany) and were used for experiments for a maximum of four weeks. Bacterial suspension including 0.3% bovine serum albumin (BSA; A6588; VWR International GmbH, Germany) concentration was prepared as described previously [34].

Surface preparation

Commercial picker fingers (20 mm bore diameter, material thickness: 17.5 mm front, 19 mm rear; total length: 97.5 mm, effective length: 90 mm; 7.5 mm protrusion to the outside (EAN: 04032966060909; Westfalia Werkzeug company GmbH & Co KG, Germany)) were used.

They were cleaned according to DIN EN 13697:2019–10 by setting them 60 min inside a 5% Decon90 (Decon Laboratories Ltd., United Kingdom) solution for decontamination. After rinsing with distilled water, they were immersed in 95% propanol (Carl Roth GmbH, Germany) solution for 15 min for sterilization and washed with distilled water, air dried in a biosafety cabinet, and subsequently autoclaved at 121 °C. Picker fingers were either stored in closed plastic bags at room temperature to be used directly for the experimental setup or were coated with nanoscale silicon dioxide. This coating was performed by Nanopool GmbH, Germany, which used its commercial product Liquid Glass Metal to apply a nanoscale layer of silicon dioxide to the picker fingers. New picker fingers were used for each experiment.

Study design

Bacterial attachment, growth and detachment of target organisms were investigated for picker fingers in regard to coating. One experiment with three uncoated and three coated picker fingers was carried out three times per approach.

Attachment

Two types of attachment were simulated at ambient temperature (25 ± 5 °C). 1) Picker fingers were immersed for one minute in 20 ml of bacterial suspension with an approximate concentration of 1.0×10^8 cfu/ml in a centrifuge tube (50 ml, TPP Techno Plastic Products AG, Switzerland) and briefly vortexed to allow complete wetting of the surface to simulate the spilling of potentially contaminated fluids in the slaughterhouse. Picker fingers were tapped three times per third on the rim of a glass dish to remove excess fluid. 2) The forces acting on the picker fingers during defeathering of the poultry were simulated with a silicon brush immersed in bacterial suspension of a concentration of 1.0×10^8 cfu/ml by passing it three times lengthwise over the third of the picker finger with constant pressure. After each third, the brush was immersed again and the process repeated until the picker finger was completely covered.

Proliferation

Growth of the target organisms on picker fingers were observed for eight hours at 30 °C. The eight-hour period was chosen to represent a work shift in slaughterhouses. Picker fingers were immersed for one minute in a gently vortexed 20 ml bacterial suspension with approx. 1.0×10^5 cfu/ml. Excess liquid was removed by tapping the picker fingers three times per third on the rim of a glass dish. All picker fingers were each placed individually upright in centrifuge tubes so that they almost completely sealed the tube and guaranteed a relative humidity of min. 70%. Tubes with picker fingers were placed upright into a polypropylene container (6.0 l; Sunware B.V., Netherland) with an attached but not closed lid. One such container comprising each three coated and three uncoated picker fingers was examined every hour.

Detachment

Detachment of bacteria was examined for water and detergent foam. Picker fingers were immersed in a 20 ml bacterial suspension (approx. 1.0×10^8 cfu/ml) for one minute. After removal of excess liquid by tapping picker fingers were immersed for three seconds in a centrifuge tube containing 30 ml of 79–82 °C distilled water. Afterwards, excess liquid was removed again by tapping the edge of a glass dish one time per third of picker finger.

A 0.5% detergent solution of a sodium hydroxide-based protein and fat solving detergent (Eiweiß-Fettlöser flüssig, Ernst GmbH & Co. KG, Germany) was prepared to test the detachment of bacteria with foam on the different surfaces. A volume of 7.5 ml was transferred into a centrifuge tube and shaken manually for 10 s to produce a stable foam. The picker fingers were immersed for one minute in 20 ml bacterial suspension (approx. 1.0×10^8 cfu/ml) in a centrifuge tube, then excess liquid was removed as described above and picker fingers were transferred to the centrifuge tube containing the freshly prepared foam. After five minutes exposure time, picker fingers were transferred for three seconds into a centrifuge tube filled with 30 ml of 79–82 °C distilled water finally freed from excess water by tapping each third of the picker finger on the rim of a glass dish.

Recovery and enumeration of bacteria

The bacteria were recovered following the described treatment, based on the method of Arnold et al. [41], as follows: the picker finger was cut off after the third rib from the terminal end with sterile scissors (see Fig. 1), transferred to centrifuge tube filled with 10 ml sodium chloride peptone solution and vortexed for 15 s at 2500 rpm (shaker RS-OS 5, Phoenix Instrument GmbH, Germany). Subsequently, decimal dilutions were spread on PC agar plates for enumeration and incubated at 37 °C for 24 h. Grown colonies were counted.

Data analysis

All bacterial counts were $\log_{10}$ transformed for statistical analysis. For every experimental day, the initial bacterial concentration was determined for further calculation of attached, multiplied, and detached bacteria because of slight day-by-day differences. The average values of three replicates were taken for bacterial attachment. From this, the mean differences between the numbers of bacteria attached and recovered were calculated from three picker fingers per replicate. Since the value represents the number of bacteria that were unable to attach to the surface the results are expressed as reduced attachment. Bacterial growth was calculated as the average of three replicates from the differences of three picker fingers at a given time minus the bacterial count at time point 0. The bacteria detached from the surfaces were calculated as the average of three replicates from the mean differences of the applied and recovered bacterial counts of three picker fingers per replicate according to the respective cleaning method. Differences between uncoated and coated discs per treatment (attachment/detachment) or per time point (growth) were statistically analyzed by unpaired t test at an alpha level of significance of 0.05. Data were mainly distributed normally as revealed by Shapiro–Wilk test. All statistical analyses were executed by Prism 9 (GraphPad Software, LLC, USA).

Fig. 1 Example of a rubber picker finger as used in experimental setups. Arrow marks the cutting edge for bacterial recovery

Results

Attachment

The immersion of picker fingers resulted in mean reduced attachment of 1.047 $\log_{10}$ cfu on uncoated and 0.963 $\log_{10}$ cfu on coated surface for *S.* E. ($P=0.130$). For *E. coli*, a similar slight but statistically significant difference was detected ($P=0.047$) with an average of 1.172 $\log_{10}$ cfu on uncoated and 1.029 $\log_{10}$ cfu on coated picker fingers (see Fig. 2A).

When the picker fingers were brushed with *S.* E. suspension, a similar number of bacteria were unable to attach on uncoated (0.979 $\log_{10}$ cfu) and coated (0.988 $\log_{10}$ cfu) picker fingers ($P=0.961$). The same effect was observed for *E. coli* ($P=0.562$) with an average of 0.892 $\log_{10}$ cfu unattached bacteria on uncoated picker fingers compared to 0.871 $\log_{10}$ cfu on coated ones (see Fig. 2B).

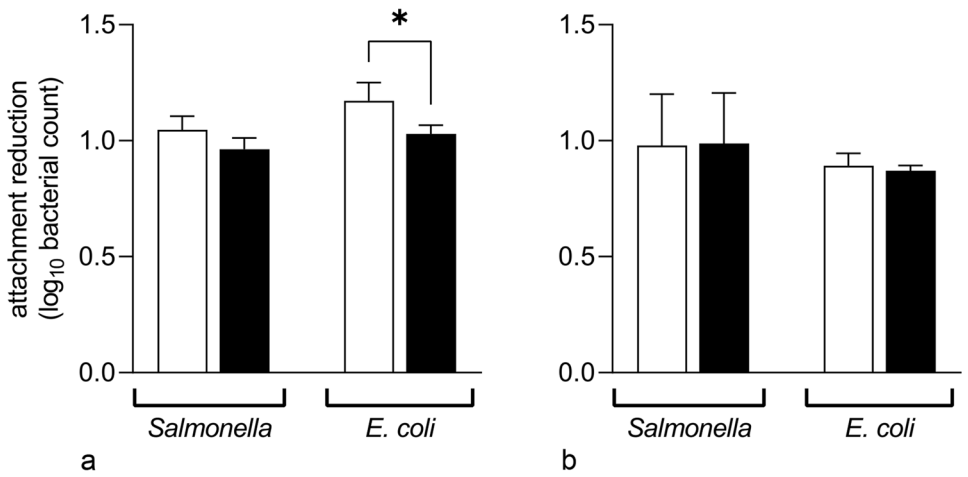

Fig. 2 Reduction of *S.* E. and *E. coli* attachment after application by immersing (**A**) or by brushing (**B**) on coated (black) and uncoated (white) rubber picker fingers. The mean values ± standard deviation from three replicates are shown

Proliferation

Similar growth was observed for both target organisms irrespective of surface modification. Starting with a concentration of 1.0×10^5 cfu/ml, bacteria grew more than four log within the observed eight hours. Neither bacterial count of *S.* E. nor of *E. coli* differed at any hour regarding the coating to a statistically significant extent (see Fig. 3, Table 1).

Detachment

By cleaning the surfaces inoculated with *S.* E. using hot water, an average of 3.652 $\log_{10}$ cfu detached from uncoated picker fingers and a similar amount of 3.578 $\log_{10}$ cfu could be removed from the coated ones ($P = 0.876$). A similar result was observed for *E. coli*, with an average detachment of 3.893 $\log_{10}$ cfu from uncoated and 3.684 $\log_{10}$ cfu from coated picker fingers ($P = 0.620$) (Fig. 4A).

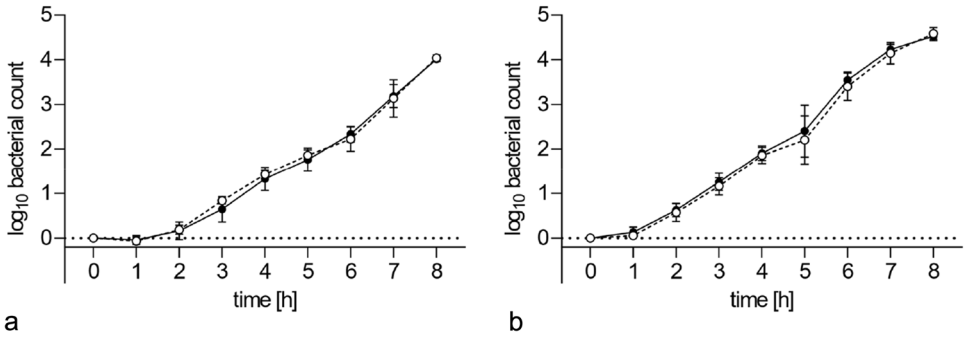

Fig. 3 Bacterial growth of *S.* E. (**A**) and *E. coli* (**B**) on coated (●) and uncoated (○) rubber picker fingers. The mean values ± standard deviation from three replicates are shown

Table 1 Mean values (± SD) from three experiments of bacterial growth ($\log_{10}$ cfu; difference to hour 0) of *S.* E. and *E. coli* on uncoated and silicon-coated rubber picker fingers ($n = 3$ each) over a period of eight hours are shown, starting concentration: approx. 1.0×10^5 cfu/ml

Hours	*S.* E		*E. coli*	
	Uncoated picker fingers	Coated picker fingers	Uncoated picker fingers	Coated picker fingers
0	0	0	0	0
1	− 0.060 (± 0.089)	− 0.038 (± 0.0977)	0.060 (± 0.0720)	0.134 (± 0.115)
2	0.189 (± 0.099)	0.165 (± 0.196)	0.575 (± 0.203)	0.625 (± 0.148)
3	0.835 (± 0.071)	0.646 (± 0.281)	1.161 (± 0.194)	1.260 (± 0.191)
4	1.427 (± 0.089)	1.326 (± 0.260)	1.859 (± 0.181)	1.909 (± 0.162)
5	1.862 (± 0.120)	1.761 (± 0.259)	2.205 (± 0.541)	2.406 (± 0.583)
6	2.228 (± 0.274)	2.336 (± 0.175)	3.400 (± 0.305)	3.545 (± 0.182)
7	3.136 (± 0.419)	3.189 (± 0.257)	4.147 (± 0.240)	4.231 (± 0.114)
8	4.041 (± 0.071)	4.012 (± 0.044)	4.589 (± 0.133)	4.527 (± 0.101)

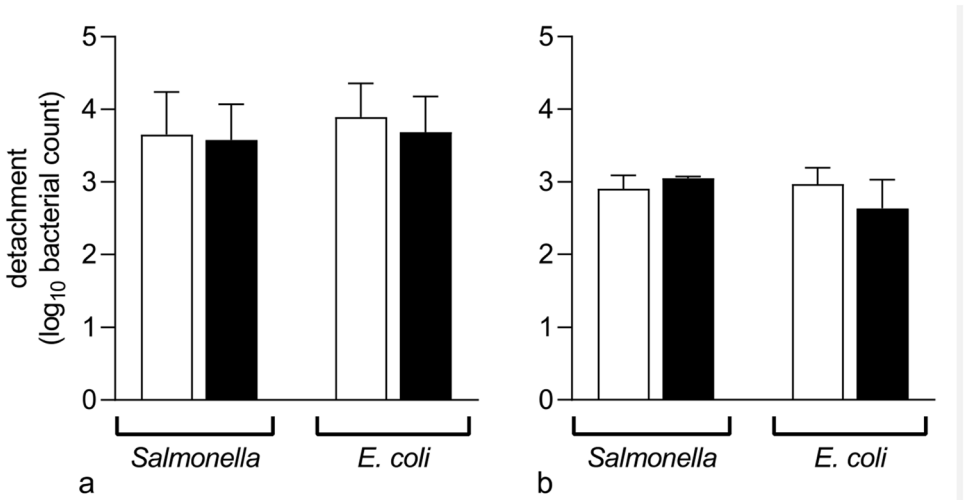

Fig. 4 Detachment of *S.* E. and *E. coli* by water (**A**) or solvent (**B**) from coated (black) and uncoated (white) rubber picker fingers. The mean values ± standard deviation from three replicates are shown

Using detergent foam, 2.904 $\log_{10}$ cfu *S.* E. were removed from uncoated and 3.046 $\log_{10}$ cfu from coated picker fingers ($P=0.260$). Comparable results were also observed for *E. coli*: an average of 2.970 $\log_{10}$ cfu detached from the uncoated surface and 2.635 $\log_{10}$ cfu from the coated surface ($P=0.272$) (Fig. 4B).

Discussion

Attachment

In this study, *S.* E. and *E. coli* tend to attach less to uncoated surfaces of rubber picker fingers than to silicon dioxide coated ones. It was found that this slight difference was statistically significant for *E. coli* after simulated contamination by immersion. This was unexpected from previous results for stainless steel [34] but may be explained by the characteristic of the rubber picker finger material itself. Arnold et al. [42] reported that compared to other material like stainless steel, rubber picker fingers exhibited a reduced bacterial adherence when they were brand-new and superficially unchanged. Due to the accompanying surface' structure alteration induced by the applied silicon dioxide, the coating might reduce this immanent material effect and, thus, lead to the observed results. However, not only the picker finger itself, but also the defeathering in the plucking machine can have an influence on bacterial attachment. This was tried to be imitated in the present study by brushing. It should be noted that the distribution pattern of microorganisms during the plucking is influenced by the movement of the picker fingers and thus depends on the type of machine, which has implications for the extent of cross-contamination of the defeathering process. Allen et al. [40] found that on poultry inoculated with *E. coli*, fewer marker organisms were found on carcasses defeathered by a contrarotating machine (mean $\log_{10}$ 5.23 ± 0.09 cfu/ml carcass rinse for single pass) than on the animals plucked by the disc machine (mean $\log_{10}$ 2.43 ± 0.41 cfu/ml carcass rinse). In the present study, this aspect was neglected because only single picker fingers were used in the experiments. No difference was found for *S.* E. and only a tendency for increased attachment on uncoated picker fingers for *E. coli* which in turn also indicates good general resistance of the rubber picker fingers against bacterial attachment.

Proliferation

Growth of different *Salmonella* serovars on various food contact surfaces such as fiberglass reinforced plastic wall paneling, stainless steel, and acetal resin could be observed for several days [43]. Consequently, it was assumed that growth would be possible on both surfaces during the estimated period.

A reason for the observed similar growth of the two target organisms on uncoated and coated picker finger could be the bacterial cell extensions, which explore the surface microstructures like appendages and can thus attach better and multiply accordingly. Friedlander et al. [44] discovered that on polydimethylsiloxane surfaces with certain topographical surface the adhesion of *E. coli* is significantly reduced in the first two hours compared to one of the not structured, flat controls. This behavior abruptly reverses to a significantly increased adhesion at longer exposure. That can be justified on the basis of the trapped air within the nanostructured surface, which are displaced after a period, four hours, by the bacteria-containing medium allowing the bacteria to also penetrate into the crevices with the cell appendages and thus have the opportunity to form a network. The solid–liquid-air state, which is described by Cassie-Baxter [45], as on porous, heterogeneous surfaces resulting in the hydrophobicity of the surface, could have been disturbed by bacterial suspension right at the beginning in the experimental setup presented. This would have allowed the bacteria to adhere to the pits of the nanostructured surface through the appendages. This could explain why there is no difference in growth between the surfaces in both bacteria in the present study.

Detachment

Hardly any difference was found between uncoated and coated picker fingers for *S.* E. and *E. coli* by washing the inoculated picker fingers with water or foam to observe their detachment from the surface. Rather, it seems that both isolates tend to detach better with water and also with foam from uncoated picker fingers than from coated ones.

As already mentioned, the organic material produced during defeathering plays a role in cross-contamination. Organic soiling was imitated in the present study by adding BSA to the bacterial suspension. Based on DIN EN 13697 the added BSA can be considered as a high protein concentration, which has also an influence on the interaction between bacteria and surface [46]. Singh et al. [47] described two effects on nanostructured surfaces like titania films. On the one hand, there is the passivation effect, which states that the accumulation of proteins reduces bacterial adhesion and biofilm formation on nanostructured surfaces by forming a thick layer that reduces the interaction of bacteria with the nanostructured surface and inhibits bacterial adhesion. On the other hand, the surface can be significantly flattened by the protein layer, which can suppress possible effects of nanoscale morphology on bacterial adhesion and detachment. Both effects may contribute to a similar surface structure, i.e. a protein layer on which bacteria will attach finally. Therefore, detachment would rather be from the protein layer

than from the material surface itself and will, thus, result in the observed similar detachment rates. Supplementary, not only proteins themselves, but also organic material, as it is massively produced also in the mechanically operated plucking plants, influences the bacteria-surface interaction [11].

The laboratory scale experiments mimicked conditions and potential contamination routes at slaughterhouses to test the effectiveness of the coating in context of this environment. Two types of contamination routes that take place in real slaughtering processes and, therefore, impact the bacterial numbers in general were neglected in our study: the direct contact of carcasses with each other and the contamination of carcasses by other factors such as air, which seems to play a role for a contaminated following carcass and also for the surrounding environment of the respective defeathering machines and also the preceding carcasses [40, 48].

Conclusion

In the present study, the response of *Salmonella* Enteritidis and *Escherichia coli* was comparatively investigated on uncoated and nanoscale coated rubber picker fingers in different tests mimicking the slaughterhouse environment.

The applied coating was only able to affect the bacterial load marginally and should be improved to gain a higher impact. Future studies should aim to ensure that the modification of food contact surfaces is precisely matched to the substrate, application area and its environment, as surfaces are subject to considerable stress and extreme conditions. In a practical environment, where several bacterial species get onto the surface during the slaughter process, the potential for biofilm formation is high, which can be challenging for a structurally identical surface modification. The limitation of the coating can also be wear and abrasion. Substantial loss can lead to a reduction of the effect. A surface that has multiple levels of roughness or several structures at the micro- and nanoscale could be challenging for various bacterial species and end up reducing cross-contamination.

Acknowledgements The study was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme (grant number: 281C104D18). Open Access funding enabled and organized by Projekt DEAL.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability and materials All data included in this manuscript are available upon request by contacting with the corresponding authors.

Declarations

Conflict of interest The authors declare that they have known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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