CENTER for PRODUCE SAFETY

CPS 2018 RFP
FINAL PROJECT REPORT

Project Title
Non-fouling food contact surfaces – prevention of biofilm and surface-mediated cross-contamination

Project Period
January 1, 2019 – December 31, 2020 (extended to March 31, 2021)

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Objectives

1. *Evaluate the baseline non-fouling properties of FDA-approved food contact substances (FCS) using fresh produce processing conditions.*
2. *Enhance the non-fouling properties of FDA-approved FCS by topographical modification without altering the chemical composition, and identify top-performing FCS that are not fouled by Listeria monocytogenes biofilm.*
3. *Determine the compliance of top-performing FCS with current industrial sanitary design, which includes material properties and integrity, and sanitization efficiency.*
4. *Evaluate the performance of top-performing FCS in a USDA pilot plant.*

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FINAL REPORT

Abstract

Sanitary design and sanitization are critical steps to help ensure food safety and prevent pathogen cross-contamination mediated by food contact substances (FCS). In response to CPS RFP priority 1.1.3 *Lm* preventive controls and 1.3.1 Preventive controls for packing and holding operations, this project developed an applicable intervention strategy to enhance the non-fouling properties of FDA or NSF-approved FCS against *Listeria monocytogenes* biofilm.

Specifically, (1) the study evaluated non-fouling properties against *Lm* biofilm formation using substrates common in produce processing environment, including stainless steel 304 and a series of FDA or NSF-approved FCS coatings, including Dursan, chromium nitride (CrN), titanium nitride (TiN), Ni-P-polytetrafluoroethylene (Ni-P-PTFE), and Lectrofluor 641; and also evaluated plastic FCS, including ultra-high-molecular-weight polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polyoxymethylene (POM). (2) The study also evaluated the effects of surface topography on FCS non-fouling performance through simple and cost-effective modification. Stainless steel 304 topographies evaluated include bare (SS304-B), stainless steel 304 finished #4 (SS304-4), and microscale dot (SS304-Dot) or line (SS304-Line) features. Similar surface patterns and features were evaluated on plastic substrates. FCS non-fouling properties were evaluated against *Lm* in mono- and cocktail- cultures in standard media and simulated produce processing condition (lettuce juice extract with 2,000 ppm chemical oxygen demand). Dursan coating was identified to possess the best non-fouling property against *Lm* monoculture biofilm. The study also found that microflora and other bacterial species have significant impacts on *Lm* biofilm formation and non-fouling properties of FCS. The results suggested that Dursan coating has the potential ability to enhance non-fouling but other factors also appear to be significant, and warrant further study. (3) Dursan coating was considered as the top performing FCS coating, and its material properties, physical integrity, and sanitization efficiency were studied and summarized; and (4) the performance of this top-performing FCS and its sanitization was conducted and validated in bench-scale experiments under simulated industrial processing conditions. Project outcomes provide scientific information to the industry in selecting FCS modification to support sanitary design of packing, holding, and processing equipment and apparatus, coatings and coating modifications to simplify cleaning and sanitization, and to prevent pathogen attachment and biofilm formation on FCS for both new and retrofitted equipment.

Background

Prevention of FCS-mediated cross-contamination plays a critical role in ensuring food safety. FCS can foster biofilm formation, which can involve normal flora biofilm formers as well as human pathogens, particularly *Listeria monocytogenes* (*Lm*). The importance of FCS coating and surface modification has been recognized by the produce industry recently. Research studies have been reported for various food commodities; however, to date, no studies have been published that specifically address the needs for FCS in the processing, packing, holding, and transportation of fresh and fresh-cut produce. Unlike FCS use in thermal processing and drying, the fresh and fresh-cut produce sector has several unique attributes, requiring that a successful FCS must tolerate wet and cold temperatures (fresh-cut) or field handling conditions. Additionally, daily sanitization is performed during fresh-cut processing, but this is not always feasible for some applications, including packinghouse operations and harvest containers. Moreover, even with daily sanitization, hard-to-access surfaces can harbor and support *Lm* biofilm formation between ‘deep-cleaning’ cycles. Thus, technologies that prevent pathogen

fouling and biofilm formation could support sanitary equipment design to make immediate impacts on produce safety.

To fill this gap and explore an applicable postharvest preventive control mechanism, a series of FDA- or NSF-approved coatings were used to study the non-fouling properties on substrates, including stainless steel 304 and plastics (e.g., ultra-high-molecular-weight polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polyoxymethylene (POM)). The selected substrates are common in produce packinghouses, fresh-cut processing plants and equipment. The study also evaluated the effect of surface topography on FCS non-fouling performance against *Lm* in mono- and cocktail biofilms.

The goal of this study was to demonstrate the potential to enhance non-fouling properties of FCS via coating and/or topographical modifications, and validate under industrially relevant conditions.

Research Methods and Results

1. Research Methods

1.1 Topographical enhancement of FCS

Surface topographies of stainless steel 304 bare (SS304-B) were modified using a simple microfabrication method without the requirement for cleanroom access. Micro-dot and micro-line patterns were fabricated on stainless steel 304 substrates (SS304-Dot, SS304-Line) using the method shown in **Figure 1**, which includes lamination, UV exposure, development, and electroetching.

Micropatterned plastics were obtained using thermal molding with inverted SS304-Dot and SS304-Line as molds at optimized molding pressures and temperatures, listed in **Table 1**.

1.2 Chemical coatings of FCS and enhanced FCS

SS304-B, stainless steel 304 with commercial #4 finishes (SS304-4), and SS304-Dot were coated with five FDA or NSF-approved coatings, including Dursan, Ni-P-polytetrafluoroethylene (Ni-P-PTFE), Lectrofluor 641, chromium nitride (CrN), and titanium nitride (TiN). SS304-Line was coated with Dursan. All coating processes were conducted by commercial FCS suppliers: SilcoTek Corporation for Dursan, General Miniplate Corporation for Ni-P-PTFE and Lectrofluor 641, and BryCoat Inc. for CrN and TiN.

1.3 Characterization of surface properties

Surface properties evaluated included surface wettability (analyzed by contact angle), surface roughness (measured by 3D microscopy), and surface topography (characterized by electron microscopy).

1.4 Evaluation of FCS and enhanced FCS performance on *Lm* biofilm formation

1.4.1 Biofilm formation on FCS coupons

FCS substrates were precleaned and sterilized prior to biofilm formation experiments. Four bacterial species were used for the *Lm* biofim study, including *Lm*, *Escherichia coli* O157:H7 (*Ec*), *Pseudomonas fluorescens* (*Pf*), and *Ralstonia insidiosa* (*RI*). *Pf* and *RI* are indigenous microorganisms on fresh produce and were previously identified as strong biofilm formers. Monoculture (*Lm*) and cocktail cultures of *Lm* + *Ec*, *Lm* + *Pf*, *Lm* + *Ri*, or *Lm* + *Ec* + *Pf* + *Ri* suspensions were prepared in tryptic soy broth–0.7% yeast extract (TSB-YE), a commonly used media in biofilm experiments. The concentration of each species in TSB-YE was approximately 7 Log CFU/mL.

Substrates were vertically placed in the mono- or cocktail-culture suspensions and incubated at 37°C for 4 h for initial bacterial attachment, which was designed to facilitate high *Lm* biofilm development to reveal differences in fouling properties among tested surfaces. Each

substrate was removed from the suspension and rinsed with phosphate buffered saline (PBS) to remove loosely attached cells. The substrates were then transferred to fresh lettuce juice extract (LJE) with 2,000ppm COD or 10% TSB-YE and stored at 4°C for 7 days for biofilm development. The temperature and incubation time were chosen to resemble produce processing conditions and typical deep-cleaning schedules of produce processing equipment, as indicated by our industry advisors.

1.4.2 Biofilm assessment

Biofilms were determined using crystal-violet (CV) staining (total biomass) and plate counting (viable cells). Briefly, substrates with biofilm were washed with PBS and fixed using 70% ethanol. The fixed substrates were air dried and stained with CV, followed by rinsing with distilled (DI) water to remove excess staining solution. Glacial acetic acid was used to re-solubilize the bonded dye for each substrate. Absorbance of the re-suspended liquid was measured at 587 nm. Viable and culturable cells in the biofilm were quantified using selective media. Substrates with biofilm were rinsed by PBS and soaked in PBS. The biofilm was removed and suspended in PBS by ultrasonic treatment. The biofilm suspension was diluted properly and plated on selective agar plates. After incubation, the colonies were counted.

1.5 Compliance of top-performing FCS

Dursan, supplied by SilcoTek Corporation (Bellefonte, PA), was identified as the top-performing FCS coating. The project included a literature review and summary of compliance results of Dursan, which were provided by the coating supplier and published literature. Compliance results include toxicity, corrosion resistance, inertness and durability properties.

1.6 Validation of top-performing FCS in a bench-scale experiment

The *Lm* cocktail (including FS2063 (4b), FS2064 (1/2a), and FS2065 (1/2b)), two environmental bacterial species previously isolated from the Co-PI's produce processing pilot plant, *Ralstonia insidiosa* and *Brevundimonas naejangsanensis* (RB), and background microflora (UM) (collected from commercial romaine lettuce and environmental surface of a produce processing pilot plant) were used for validation of top-performing FCS treatments (Dursan).

The substrates (SS304-B, SS304-Dot, Dursan-coated SS304-B, and Dursan-coated SS304-Dot) were cultivated in 2000ppm LJE with 7 Log CFU/mL *Lm* at 25°C for 5 h. After incubation, each substrate was washed in sterilized DI water (SDW) for 30 s, and transferred to pure 2000ppm LJE, 2000ppm LJE with 5 Log CFU/mL RB, or 2000ppm LJE with 5 Log CFU/mL UM solution for biofilm formation at 25°C for 3 days. After 3-d biofilm formation, substrates were sanitized in 200 ppm quaternary ammonium compounds (BiQ, pH 6.5), or 10 ppm free chlorine (FC, pH 6.5) for 30 s, and then soaked in SDW for another 30 s. SDW was used as control. Bacterial biofilm populations were enumerated by plate count on tryptic soy agar (TSA) and Harlequin® *Listeria* Chromogenic agar (HLCA) plates.

2. Research Results

2.1 Characterization of surface properties of FCS

2.1.1 Surface properties of stainless steel 304

The surface property characterization of SS304 substrates is presented in **Tables 2** and **3**, and **Figures 2** and **3**. Micropattern dimensions for SS304-Dot were 39.4 ± 4.4 µm in diameter and 1.8 ± 0.38 µm in height, and the interpillar spacing was 8.5 ± 1.5 µm; those for SS304-Line were 47.5 ± 5.0 µm in diameter and 5.0 ± 0.01 µm in width.

Figure 2 shows optical images and color topographic renderings of SS304-B, SS304-4, SS304-Dot, and SS304-Line surfaces. The SS304-B surface is flat and smooth with a roughness of 0.041 ± 0.017 µm (Table 3 and Figure 2A). The SS304-4 has a linear texture with

arrays of short grooves and asperities, which are finely uniform and directional in appearance, and with an increased surface roughness of 0.118 ± 0.031 µm (Table 3 and Figure 2C). The surface of SS304-Dot/Line substrates (Figure 2E/2G) shows successful dot/line pattern generation and a high degree of pattern uniformity, with high fidelity of pattern features and dimensions to the CAD design and photoresist mask. The average roughness for SS304-Dot and SS304-Line is 1.773 ± 0.384 µm and 52.762 ± 4.6 µm, respectively, both higher than for SS304-B (Table 3).

Figure 3 presents the contact angle testing results of SS304 substrates. The surface of the unmodified SS304-B substrate is slightly hydrophobic, with a contact angle of 96 ± 1.1°. The two topographic modifications are significantly more hydrophilic, with SS304-4, SS304-Dot, and SS304-Line having contact angles of 60 ± 0.2°, 80 ± 1.3°, and 68.7 ± 2.6°, respectively. Coating treatments changed surface hydrophobicity/wettability of the SS304 substrates. Lectrofluor 641 significantly increased hydrophobicity of SS304-B, SS304-4, and SS304-Dot, with contact angles higher than those of the corresponding uncoated substrates by 19°, 62°, and 40°, respectively. Ni-P-PTFE hydrophilized the substrates, with the contact angles decreasing by 31°, 8°, and 25° for SS304-B, SS304-4, and SS304-Dot, respectively. Coating with CrN increased hydrophobicity on SS304-B and SS304-4 but increased hydrophilicity on SS304-Dot. TiN enhanced hydrophilicity on SS304-B and SS304-Dot, while SS304-4 became more hydrophobic. Dursan increased the wettability of SS304-B, SS304-4, and SS304-Line, but slightly decreased the wettability on SS304-Dot.

2.1.2 Surface properties of plastics

Table 1 shows the optimized thermal molding conditions for dot- and line-patterns on PVC, PP, PE, and POM. The surface property characterization of plastic substrates is presented in **Table 4** and **Figure 4**. The average surface roughness of the bare plastics and micropatterned plastics is shown in Table 4. The surface roughness of bare PP, PE, PVC, and POM is 12.28 ± 2.9 µm, 42.38 ± 7.9 µm, 7.36 ± 1.5 µm, and 13.54 ± 0.94 µm, respectively. The roughness of four plastic substrates after dot and line patterning via thermal molding, respectively, is consistently increased, ranging from 7.9–46.5 µm. Figure 4 presents the contact angle and wettability results. Both bare PP and PE substrates are hydrophobic, with contact angles of 102.3 ± 0.4 ° and 104.5 ± 0.4 °, respectively; bare PVC and POM are hydrophilic, with contact angles of 82.2 ± 0.6 ° and 83.0 ± 0.4 °, respectively. The modifications via dot and line patterning consistently increase the surface hydrophobicity of PP, PVC and POM, but not for PE and thus the modifications result in the wettability increment.

2.2 Evaluation of FCS and enhanced FCS performance on *Lm* biofilm formation

2.2.1 Effects of surface modification on Lm biofilm formation and top-performing FCS

Figure 5 presents the effects of surface modifications on *Lm* biofilm formation on SS304. Biofilm formation was reduced on the micropatterned surfaces of SS304-Dot and SS304-Line. Dursan-coated SS304-B and SS304-Dot showed the best non-fouling properties. Substrates coated with Ni-P-PTFE also showed reduced biofilm formation on SS304-B compared to the uncoated control. In contrast, other coatings (i.e., CrN, TiN, and Lectrofluor 641) did not show good resistance to *Lm* biofilm formation. In addition, the synergistic effects of surface topography and coating on improving non-fouling properties were found in Ni-P-PTFE coated SS304-B and SS304-4 substrates. Dursan was identified as the best FCS coating for subsequent analysis.

Figure 6 shows the impact of wettability on *Lm* biofilm formation on SS304 substrates. The surface wettability significantly affected biofilm formation, however, no obvious trend between them was found. Therefore, wettability does not appear to be a reliable indicator of non-fouling properties against *Lm* biofilm.

Figure 7 shows the non-fouling mechanism of Dursan-coated SS304-Dot against *Lm* biofilm. On the unmodified SS304-B (Figure 7A), the bacteria easily attach on the SS304 surface and the biofilm matures over time. Previous studies showed superior anti-protein fouling properties, and protein was found in many biofilm studies to have a significant impact on biofilm development. In Figure 7B, Dursan-coated SS304-Dot can inhibit the adhesion of bacteria and development of biofilm and achieve the fouling resistance.

2.2.2 Effect of cocktail species on Lm biofilm formation on top-performing FCS

Cocktail species significantly impacted *Lm* biofilm formation on SS304. *Lm* fouling and biofilm development were likely to increase when cultured with other species (*Ec*, *Ri*, and/or *Pf*). This was observed in both standard media (**Figure 8**) and simulated processing water (**Figure 9**). Dursan coating showed small improvements in non-fouling properties on SS304-4, SS3044-Dot, and SS304-Line (Figure 8E). In addition, the dot-patterned modification also showed non-fouling properties against two-strain cocktail of *Lm* + *Ec* and the four-strain cocktail. Similarly, Figure 9 shows that Dursan only had small improvement against *Lm* cocktail biofilm in simulated processing water on SS304-Dot (Figure 9C and 9E). Instead of using *Lm* alone, results suggest future work should consider using cocktail biofilms in antibiofilm and antifouling studies.

2.2.3 Effects of plastics with or without surface modification on Lm biofilm formation

Figure 10 shows *Lm* biofilm formation on various plastic substrates. The surface modifications slightly reduced the *Lm* monoculture biofilm formation (Figure 10A). Cultivations of *Lm* with other species did not show any obvious trend in affecting *Lm* biofilm formation. Physical and topographical modification improved non-fouling properties on PE, POM, and PVC with dotted micropattern, and PE, PVC, and POM with line micropatterns. However, no obvious trend was observed across all plastic substrates.

2.3 Compliance of Dursan coating

Dursan, offered by SilcoTek, is a proprietary, patented, NSF International–certified (NSF, 2021) and FDA-compliant food equipment material (SilcoTek Corporation, 2021; FDA, 2018). Dursan is a SiO_2-based coating, with the surface functionalized by alkyl groups to render the coating chemically inert and hydrophobic. Substrates, including stainless steel, alloy, ceramic, and glass, are coated with Dursan by using a chemical vapor deposition (CVD) process, which enables the coating to be highly tolerant and durable under extreme temperatures and pressures. **Table 5** lists specifications of Dursan coating (SilcoTek Corporation, 2020a).

2.3.1 Non toxicity–biological compliance data

Dursan-coated samples were evaluated for potential cytotoxic effects using an in vitro mammalian cell culture test (North American Science Associates, Inc.). No cytotoxicity or cell lysis was observed, indicating that Dursan is biologically safe. Dursan has been certified to meet the United States Pharmacopeia Convention (USP) Class VI standards for biocompatibility.

2.3.2 Corrosion resistance

The corrosion resistance of Dursan coating was tested by SilcoTek Corporation (2020b). In this section, substrates were tested by SilcoTek Corporation for other manufacturing environments, but not related to or proposed in this CPS project.

A cycle corrosion test (ASTM G85-A2) was performed using acidified salt spray. After 8,064 hours of acidified salt spray treatment, Dursan-coated stainless steel 316L (316L) was not affected and the coating provided excellent protection on stainless steel in a salt spray environment compared with uncoated 316L (**Figure 11A**). Notably, Dursan-coated 316L was completely unaffected by the acidified salt even after 168 days (**Figure 11B**).

A laboratory immersion test (ASTM G31) was performed at room temperature using 6M hydrochloride (HCl) and 15% bleach. As shown in **Figure 12**, the corrosion rates of Dursan-coated 316L, C276, and C22 in 6M HCl were 1.84, 1.21, and 1.41%, much smaller than those of Monel and Inconel. The results indicate that Dursan coating and Hastelloy provide an excellent barrier to prevent corrosion from HCl (Figure 12). In 15% bleach, the corrosion rate of Dursan-coated 316L was notably lower than that of uncoated 316L (**Figure 13**), indicating Dursan provided stable protection from bleach corrosion. The results demonstrate that Dursan coating is especially useful in biomedical and pharma applications where bleach is commonly used.

2.3.3 Inertness

The SiO_2-based chemical framework in Dursan is a robust and inert barrier suitable for several process environments (Patterson et al., 2015). Dursan exhibited bio-inertness, preventing non-specific protein adsorption, which was reported by Vaidya et al. (2016). Coated substrates were tested by Vaidya et al. (2016) for other manufacturing environments, but not related to or proposed in this CPS project.

After exposure to 1% of bovine serum albumin (BSA) and immunoglobulin G (IgG), both Dursan-coated and uncoated stainless-steel sensors showed protein adsorption (**Figure 14**A and 14C). The frequency drop for the uncoated SS sensor was 4-fold lower than that for Dursan-coated sensor. When the sensors were rinsed with WB1 (i.e., the buffer with nonionic surfactant), a slight increase of frequency was observed for uncoated SS sensor while the frequency for Dursan-coated SS sensor reverted back to the baseline (Figure 14B and 14D). These observations indicated that it is much more effective to remove the adsorbed BSA from the Dursan-coated surface than from the uncoated SS surface.

2.3.4 Durability properties

The durability/mechanical wear of Dursan coating was evaluated by Vaidya et al. (2016). Dursan-coated and PTFE-coated (AF-1600) QCM-D sensors were cleaned by sonicating in ethanol for 10 min and drying with nitrogen gas. Images obtained using an optical microscope are displayed in **Figure 15**. No visible changes were detected in Dursan-coated sensor (Figure 15A and 15B). However, some of the AF-1600 coating was delaminated from the sensor after cleaning/processing (Figure 15C and 15D). The observations demonstrated the mechanical wear resistance of Dursan coating. PTFE and QCM-D sensors were tested by Vaidya et al. (2016) for other manufacturing environments, but not related to or proposed in this CPS project.

2.4 Validation of top-performing FCS

As shown in **Figure 16**, *Lm* biofilm populations on Dursan-coated SS304-B were significantly lower (over 0.6 Log) than *Lm* levels on SS304-B in 2000ppm LJE. However, there was no significant difference in *Lm* biofilm populations between the coated and uncoated SS304-Dot. Also, there was no significant difference in *Lm* biofilm populations between SS304-B and Dursan-coated SS304-B after co-incubation with RB and UM bacteria.

Biofilm populations of *Lm* on tested stainless-steel coupons were reduced 1~2.2 log after sanitation using 200 mg/L BiQ or 10 mg/L free chlorine for 30 s (**Figure 17**). The efficacy of sanitation using BiQ or free chlorine on *Lm* could be significantly affected by the cocktail biofilm formed with RB and UM. Co-incubation with UM bacteria in lettuce juice resulted in increased efficacy of sanitation using BiQ on *Lm* biofilm formed on stainless-steel coupons (0.9 log higher reduction).

The results validated that 1) without the presence of other species, Dursan coating can enhance the non-fouling properties of SS304, and exhibited benefits for BiQ sanitizer to remove biofilm; 2) other bacterial species affect *Lm* biofilm formation and sanitization; 3) different sanitizers display various efficacies for biofilm removal.

Outcomes and Accomplishments

1. Baseline nonfouling properties of common food contact surfaces (stainless steel [SS304 and SS304-4] and plastics [PE, PP, PVC and POM]) were established against *Listeria monocytogenes* (*Lm*) biofilm.
2. Baseline nonfouling properties of FDA- or NSF-approved FCS coatings, including Dursan, CrN, TiN, Ni-P-PTFE, and Lectrofluor 641 were established against *Lm* biofilm.
3. A simple and cost-effective process was developed to fabricate micropatterns (dot/line) on SS304 and plastic surfaces.
4. Impacts of surface topography, surface chemistry, and biofilm growth condition were evaluated on *Lm* biofilm formation to identify the top performing FCS.
5. Non-fouling properties were systemically evaluated against *Lm* monoculture biofilm and cocktail biofilm on the top-forming FCS.
6. Performance of top-performing FCS were validated in both the PI and Co-PI's labs.
7. Sanitation efficiency on different substrates was evaluated against *Lm* biofilm.

Summary of Findings and Recommendations

The key take-home messages from this study are as follows:

- Surface chemistry (coating) has a significant impact on *Listeria monocytogenes* (*Lm*) biofouling and biofilm development.
- Surface topography has impacts on *Lm* biofouling, and the impact is limited at the pattern scale of ~50 micron tested in this study.
- Surface wettability is not a good indicator of FCS non-fouling properties against *Lm* biofilm, as both hydrophilic and hydrophobic coating showed antifouling property.
- Dursan coating appears to have the best non-fouling properties against *Lm* monoculture biofilm among the FCS coatings tested.
- Normal microflora from produce and processing environment have significant impact on *Lm* biofouling and biofilm development and FCS non-fouling properties, including Dursan coating.
- *Lm* risk could be commodity-specific (different microflora) and processing line-specific (different surface properties).
- The presence of normal microflora appears to protect *Lm* in the cocktail biofilm against sanitizer and reduce sanitation efficiency.
- More studies are needed to develop universally effective risk mitigation technologies against *Lm* biofilm with the presence of complex microflora.

APPENDICES

Publications (in preparation)

1. Tingting Gu, Apisak Meesrisom, Yaguang Luo, Quynh N Dinh, Sophia Lin, Manyun Yang, Arnav Sharma, Ruogu Tang, Jinde Zhang, Zhen Jia*, Patricia D. Millner, Arne J. Pearlstein, Boce Zhang*. *Listeria monocytogenes* biofilm formation as affected by stainless steel surface topography and food contact substances. Food Control, 2021 (*Submitted*: Favorable review comments received; manuscript currently being revised.)
2. Tingting Gu, Yaguang Luo, Apisak Meesrisom, Quynh N Dinh, Sophia Lin, Manyun Yang, Arnav Sharma, Ruogu Tang, Zhen Jia, Patricia D. Millner, Arne J. Pearlstein, Boce Zhang*. Managing *Listeria monocytogenes* biofilm formation on stainless steel with surface topography, surface chemistry, and cocktail symbiotic bacteria. *In preparation*
3. Tingting Gu, Apisak Meesrisom, Yaguang Luo, Quynh N Dinh, Sophia Lin, Manyun Yang, Arnav Sharma, Ruogu Tang, Jinde Zhang, Zhen Jia, Patricia D. Millner, Arne J. Pearlstein, Boce Zhang*. *Listeria monocytogenes* biofilm formation on food contact plastics as affect by surface topography, surface chemistry, and cocktail symbiotic bacteria. *In preparation*
4. Ganyu Gu, Jia Zhen, Boce Zhang, Yaguang Luo, Marina Lichtenwald, Brenda Kroft, Xiangwu Nou*. Listeria monocytogenes* biofilm formation on coated and non-coated stainless-steel coupons in lettuce juice as affected by plant and environmental microbes. *In preparation*

Presentations

1. Zhang, B. 2019. Non-fouling food contact surfaces - prevention of biofilm and surface-mediated cross-contamination. CPS Research Symposium, Austin, TX
2. Zhang, B., Luo, Y. 2020. Non-fouling food contact surfaces – prevention of biofilm and surface-mediated cross-contamination CPS Research Symposium Webinar Series II.
3. Zhang, B., Luo, Y. 2021. Non-fouling food contact surfaces – prevention of biofilm and surface-mediated cross-contamination CPS Research Symposium Webinar Series V.

Budget Summary

Total research funds awarded to this project were $261,925. By the end of the project term, all funds will be expended.

Tables 1–5 and Figures 1–17

Table 1 The optimal conditions for thermal molding of micropatterned plastics

	Pressure (ton)	Temperature (ºC)
Dot-patterned		
PVC	30	60
PP	30	110
PE	30	110
POM	30	110
Line-patterned		
PVC	15	80
PP	25	80
PE	40	55
POM	25	80

Table 2 Dimension of micropatterns on SS304 substrate (μm) ± standard error (SE)

Dot pattern	Diameter	Interpillar spacing
	39.4 ± 4.4	8.5 ± 1.5
Line pattern	Diameter	Width
	47.5 ± 5.0	5.0 ± 0.01

Table 3 Surface average roughness of SS304 substrate (μm) ± SE

Substrate	SS304-B	SS304-4	SS304-Dot	SS304-Line
Average Roughness	0.041 ± 0.017	0.118 ± 0.031	1.773 ± 0.384	52.762 ± 4.6

Table 4 Average surface roughness of plastics (μm) ± SE

	PP	PE	PVC	POM
Bare	12.28 ± 2.9	42.38 ± 7.9	7.36 ± 1.5	13.54 ± 0.94
Dot	58.82 ± 8.8	69.92 ± 10.6	35.4 ± 6.7	49.62 ± 6.4
Line	45.2 ± 7.6	50.26 ± 7.9	64.36 ± 6.7	32.88 ± 5.6

Table 5 Dursan specifications

Coating structure	Functionalized silica-like coating (a-SiOX:CHY)
Deposition Process	Thermal chemical vapor deposition (not plasma-enhanced)
Maximum Temperature	500° C (inert atmosphere) 450° C (oxidative)
Substrate	Compatibility: Stainless steel, exotic alloys, ceramics Size: Up to 78” Geometry: Any shape
Typical Thickness	400 - 1600 nm
Hydrophobicity (contact angle)	>= 81°
Allowable pH Exposure	0 – 14

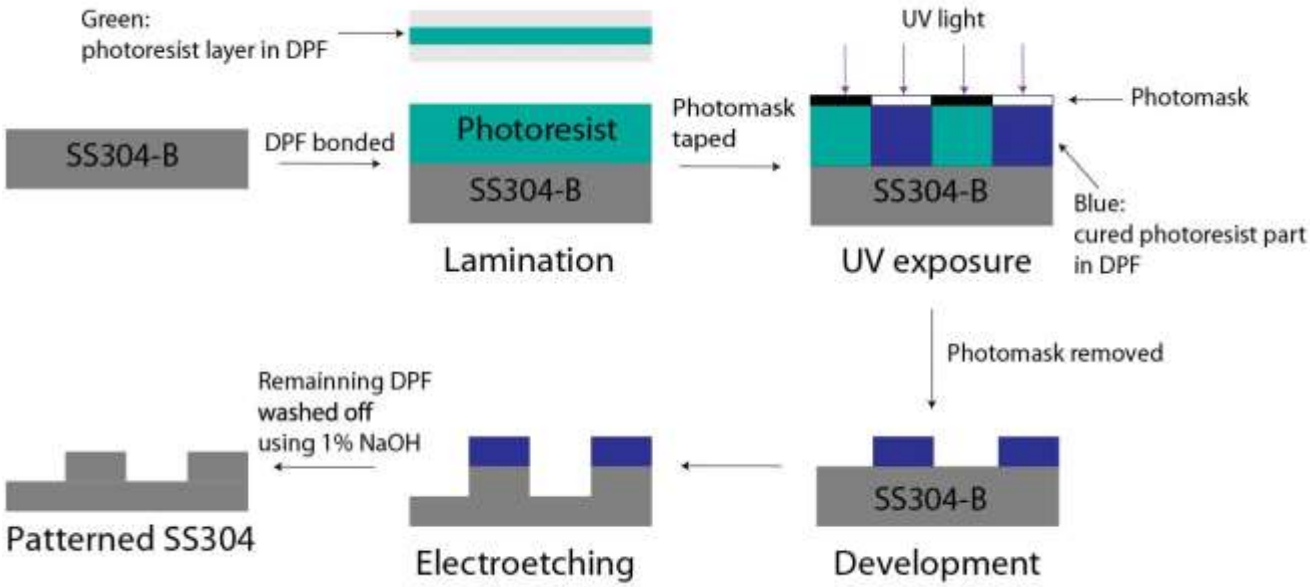

Figure 1 The fabrication process for Dot-, Inverted dot-, and Line- patterned SS304 substrates

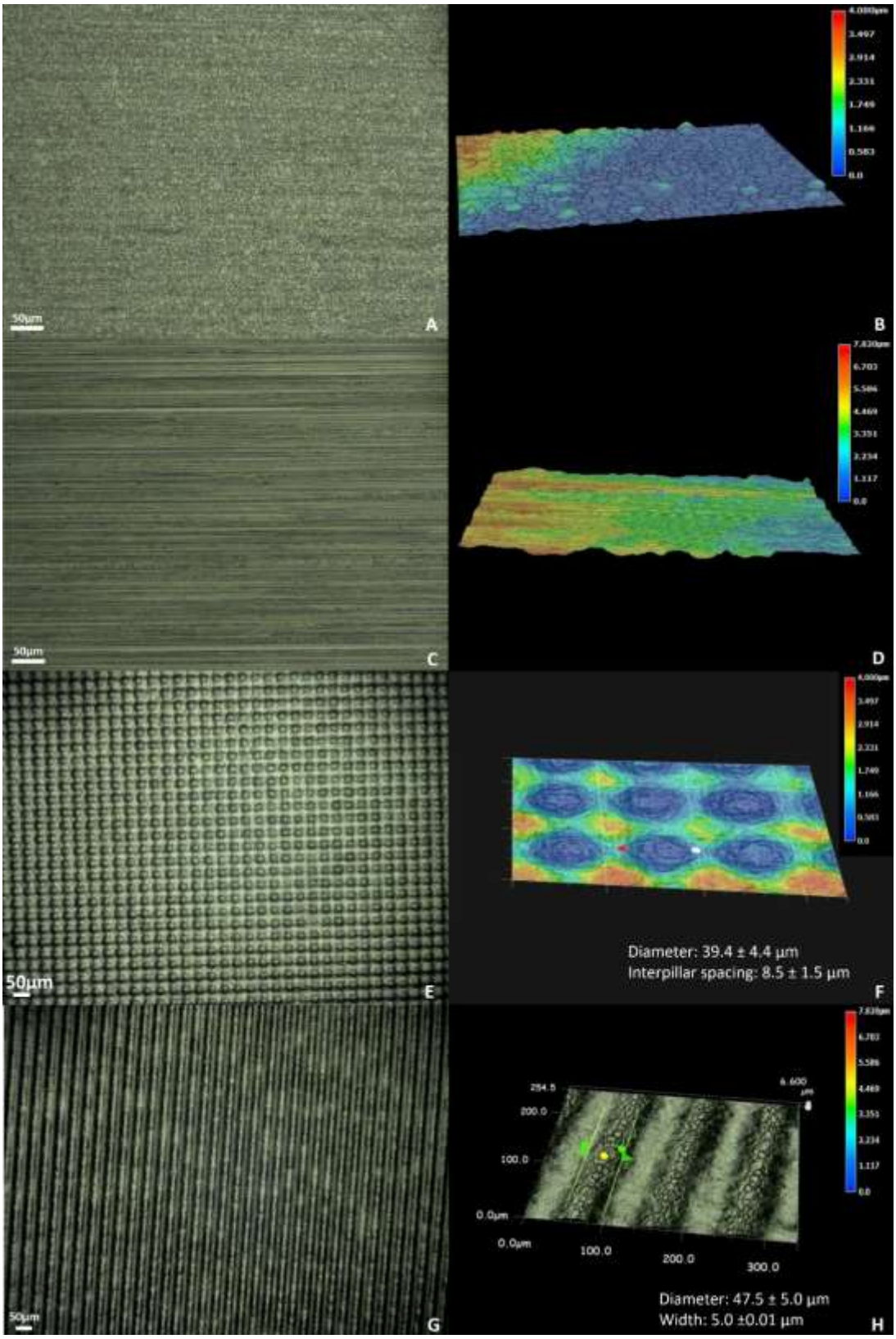

Figure 2 SS304-B surface (**A**)&(**B**) and SS304-4 (**C**)&(**D**) at 200x magnification, SS304-Dot (**E**)&(**F**) and SS304-Line (**G**)&(**H**) at 100x magnification using 3D microscope.

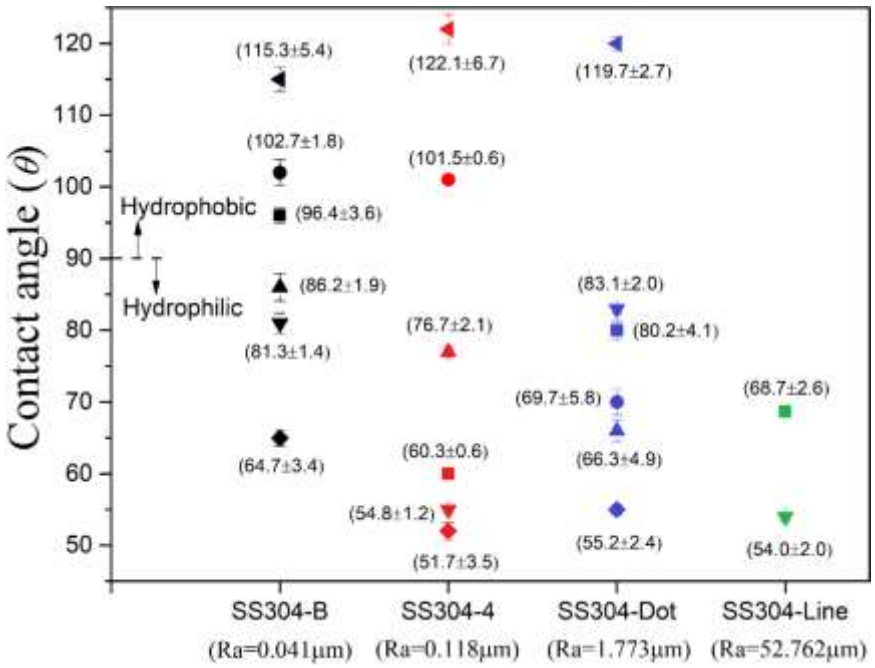

Figure 3 Effects of surface modification on the contact angle (θ) of SS304 substrates, uncoated and coated. Values are the means of 10 independent measurements ±SE. Ra is the average roughness of the native surface. Symbols: □ (uncoated); ○ (CrN); △ (TiN); ▽ (Dursan); ◇ (Ni-P-PTFE); ◁ (Lectrafluor 641).

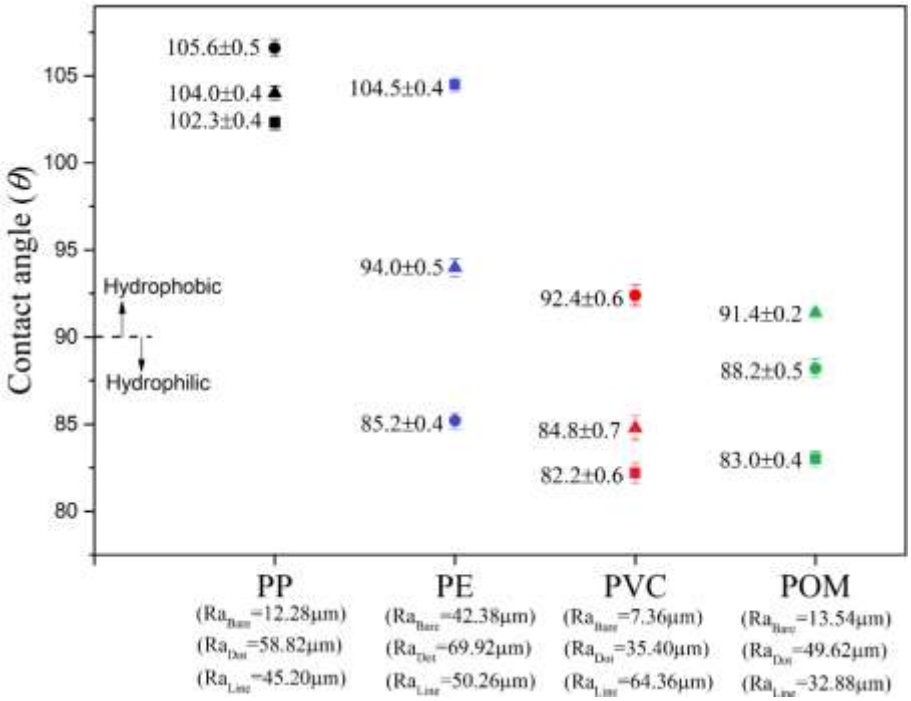

Figure 4 Effects of surface modification on the contact angle (°) of plastic substrates. Values are the means of 10 independent measurements ±SE. Ra is the average roughness of different surfaces. Symbols: □ (Bare); ○ (Dot); △ (Line).

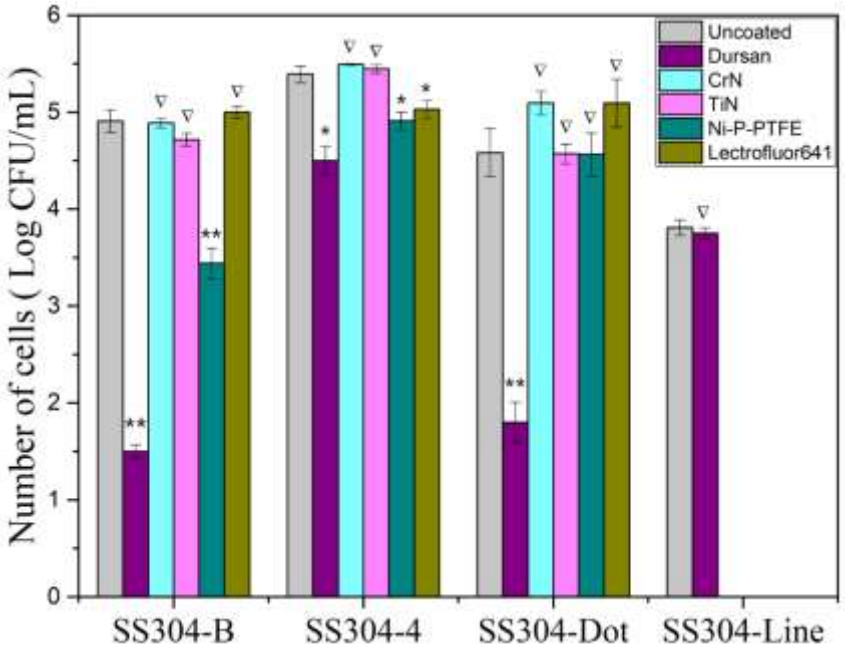

Figure 5 Effects of surface modification on mono *Lm* biofilm formation on the substrates. Error bars denote mean ± SE from five replicates. Results for coated substrates with bars bearing symbols differ significantly from their respective uncoated controls. ** $P < 0.001$; * $0.001 < P < 0.05$; ∇ $P > 0.05$.

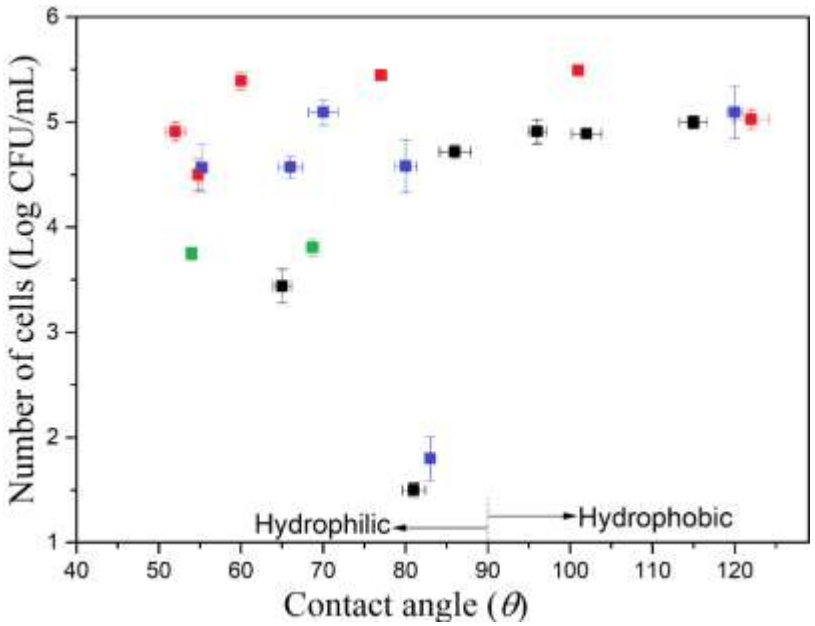

Figure 6 Effects of surface wettability on mono *Lm* biofilm formation for the four uncoated and coated SS304 substrates. Error bars denote mean ± SE of ten wettability measurements (horizontal) and five bacterial cell enumerations (vertical). Black symbol: SS304-B; red symbol: SS304-4; blue symbol: SS304-Dot; green symbol: SS304-Line.

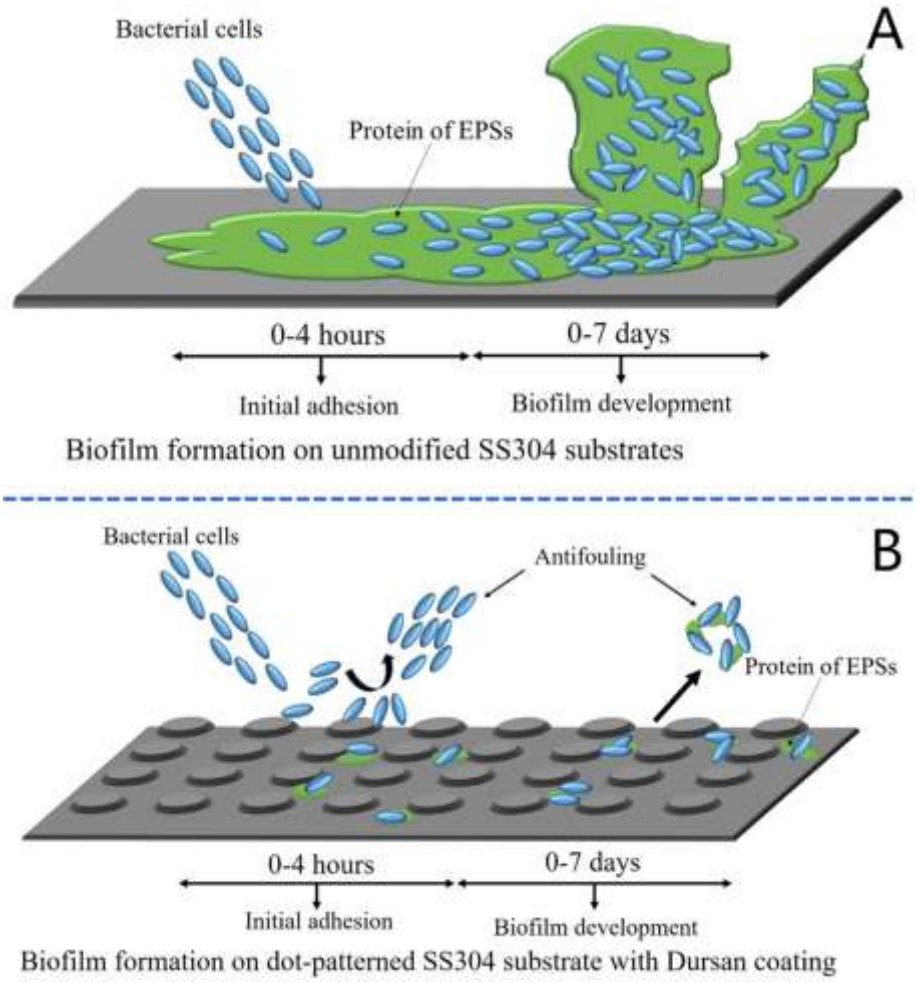

Figure 7 The process of fouling resistance against *Lm* biofilm: A) unmodified SS304-B; B) Dursan SS304-Dot.

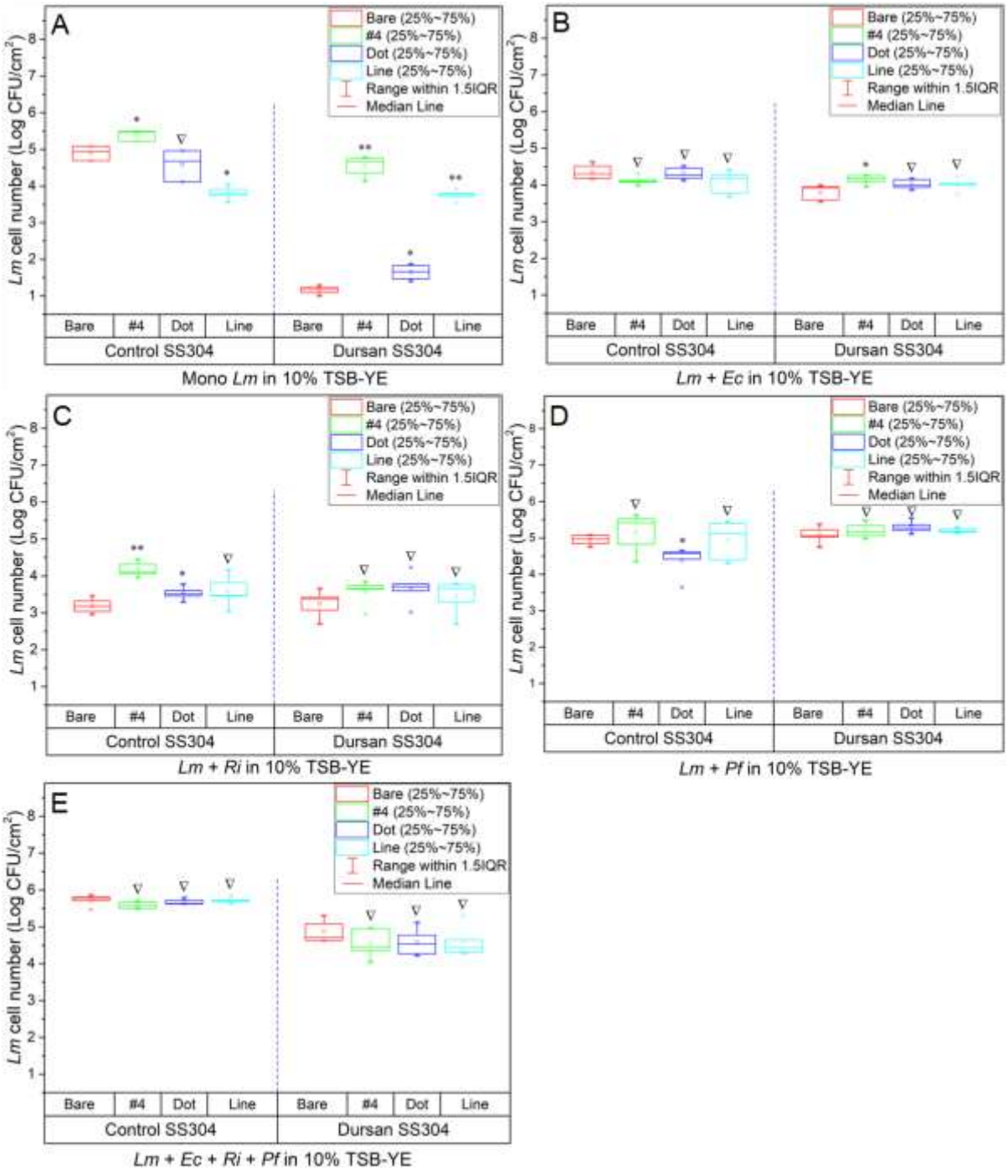

Figure 8 Effects of cocktail species on *Lm* biofilm formation on top-performing FCS in 10% TSB-YE: mono *Lm* (A), cocktail of *Lm* + *Ec* (B), cocktail of *Lm* + *Ri* (C), cocktail of *Lm* + *Pf* (D), cocktail of *Lm* + *Ec* + *Ri* + *Pf* (E). Results for topographically modified substrates with bars bearing symbols differ significantly from their respective non-topographically modified controls. ** $P < 0.001$; * $0.001 < P < 0.05$; ∇ $P > 0.05$. Note: cell counts of *Ec*, *Ri*, and *Pf* in cocktail biofilm will be available in future publication or upon reasonable request.

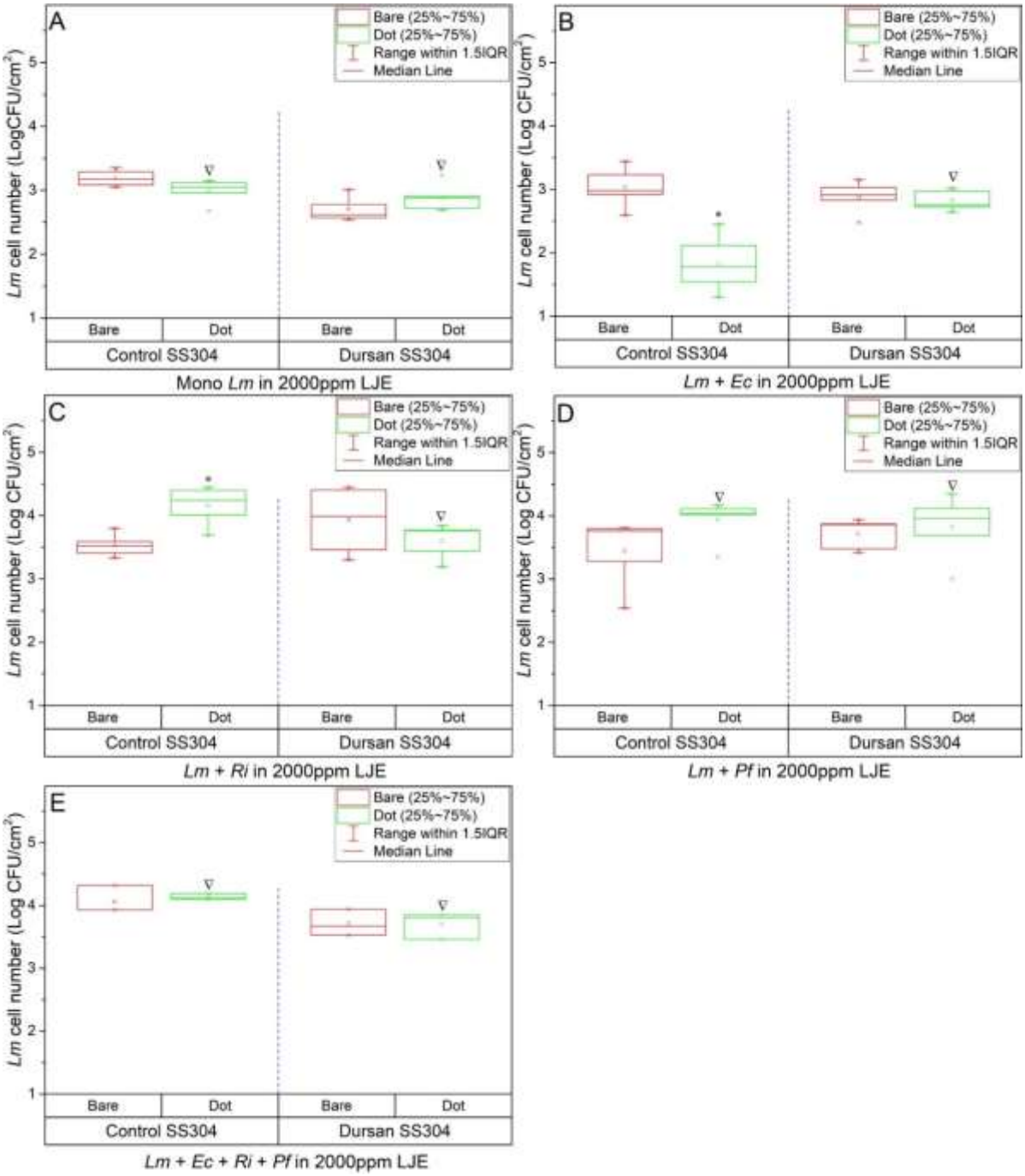

Figure 9 Effects of cocktail species on *Lm* biofilm formation on top-performing FCS in 2000ppm LJE: mono *Lm* (A), cocktail of *Lm* + *Ec* (B), cocktail of *Lm* + *Ri* (C), cocktail of *Lm* + *Pf* (D), cocktail of *Lm* + *Ec* + *Ri* + *Pf* (E). Results for topographically modified substrates with bars bearing symbols differ significantly from their respective non-topographically modified controls. ** $P < 0.001$; * $0.001 < P < 0.05$; ∇ $P > 0.05$. Note: cell counts of *Ec*, *Ri*, and *Pf* in cocktail biofilm will be available in future publication or upon reasonable request.

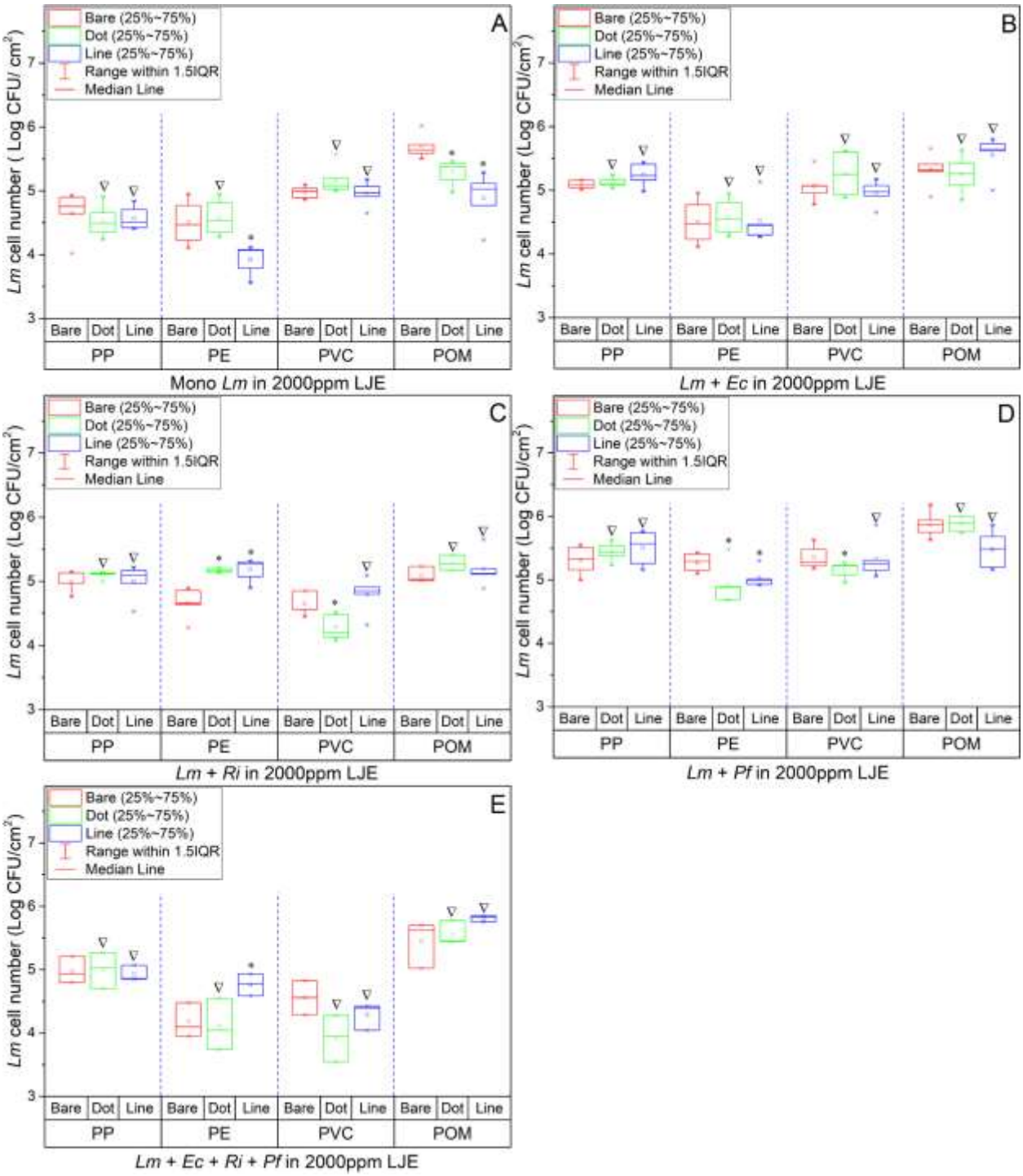

Figure 10 *Lm* biofilm development on plastics in 2000ppm LJE: mono *Lm* (A), cocktail of *Lm* + *Ec* (B), cocktail of *Lm* + *Ri* (C), cocktail of *Lm* + *Pf* (D), cocktail of *Lm* + *Ec* + *Ri* + *Pf* (E). Results for topographically modified substrates with bars bearing symbols differ significantly from their respective non-topographically modified controls. ** $P < 0.001$; * $0.001 < P < 0.05$; ∇ $P > 0.05$. Note: cell counts of *Ec*, *Ri*, and *Pf* in cocktail biofilm will be available in future publication or upon reasonable request.

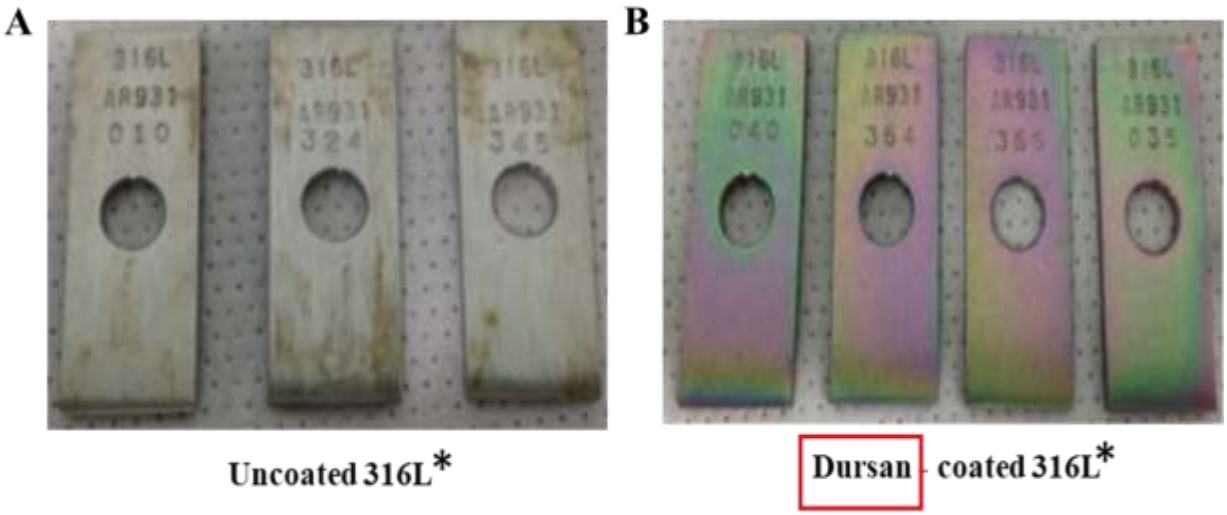

Figure 11 Salt spray analysis of 316L and Dursan-coated 316L (SilcoTek Corporation, 2020b).
* The substrate was tested by SilcoTek Corporation for other manufacturing environments, but not related to or proposed in this CPS project.

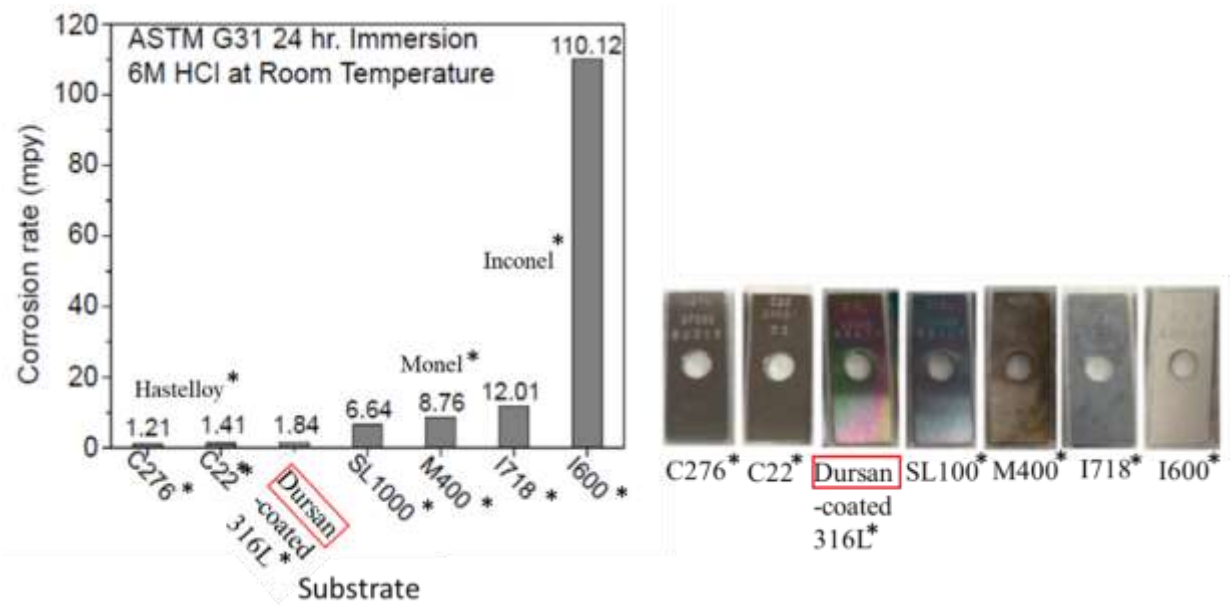

Figure 12 Hydrochloride (HCl) immersion analysis of various substrates (SilcoTek Corporation, 2020b). * These coatings/substrates were tested by SilcoTek Corporation for other manufacturing environments, but not related to or proposed in this CPS project.

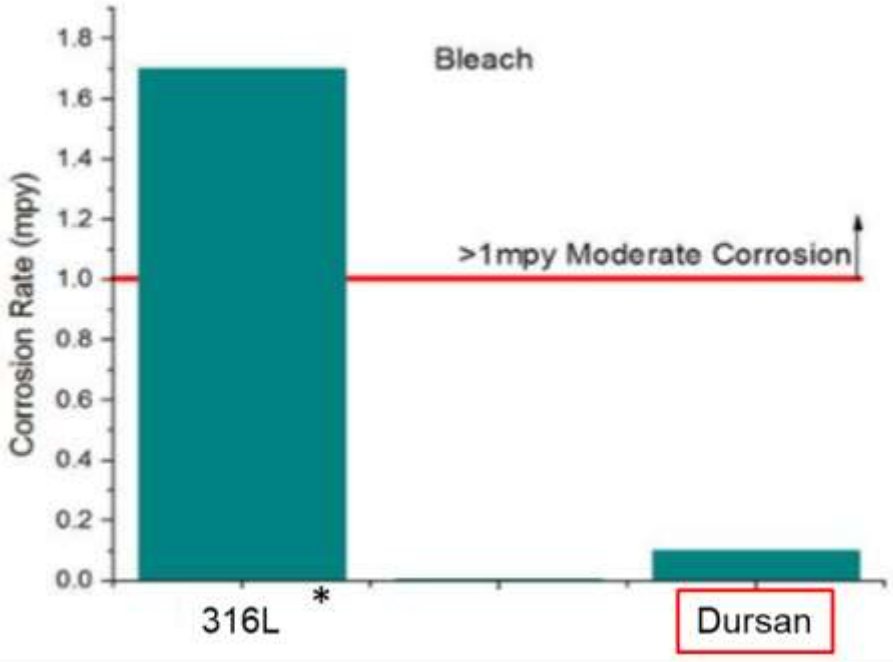

Figure 13 15% (w/w) bleach treatment analysis (SilcoTek Corporation, 2020b). * The substrate was tested by SilcoTek Corporation for other manufacturing environments, but not related to or proposed in this CPS project.

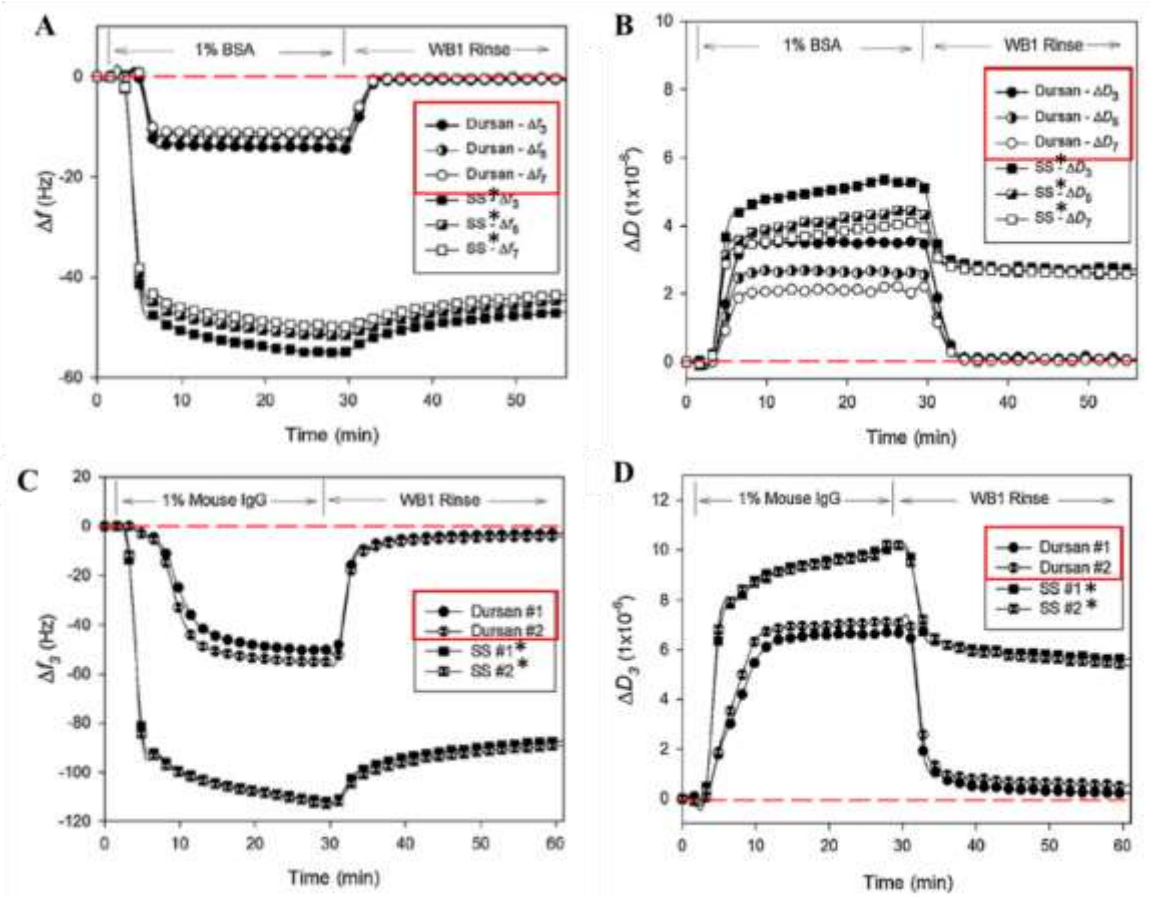

Figure 14 QCM-D profiles of sensor frequency and dissipation vs. time data at the 3rd overtone comparing adsorption of protein on Dursan-coated and bare SS316L sensors (Vaidya et al., 2016). * These substrates were tested by Vaidya et al. (2016) for other manufacturing environments, but not related to or proposed in this CPS project.

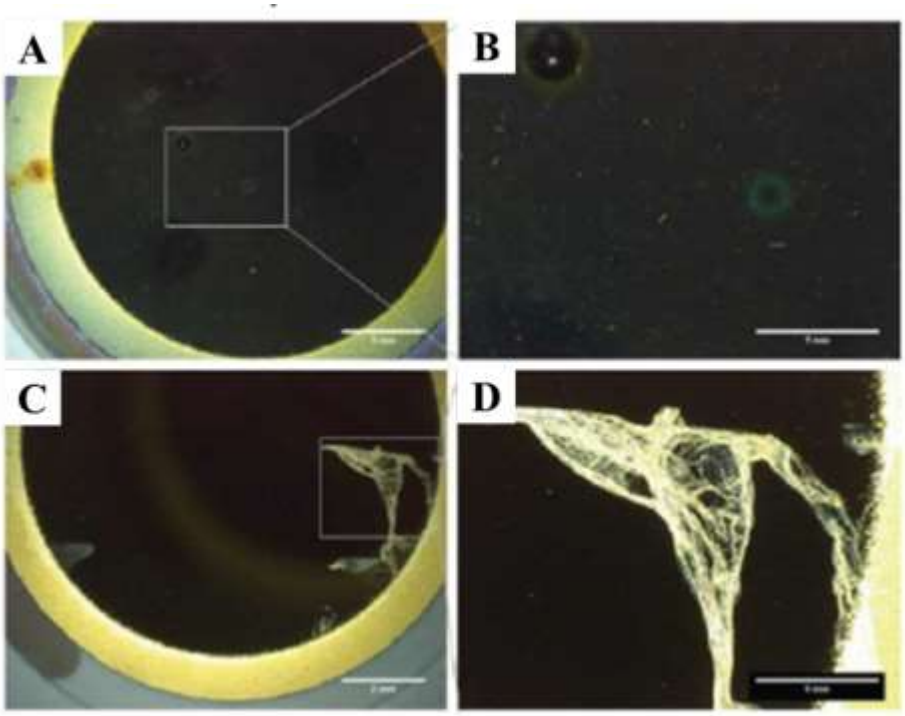

Figure 15 Optical micrographs of Dursan- and PTFE-coated QCM-D sensors before and after treatment of solvent sonication. (A)&(B): Dursan-coated sensor; (C)&(D): AF-1600-coated sensor. PTFE and QCM-D sensors were tested by Vaidya et al. (2016) for other manufacturing environments, but not related to or proposed in this CPS project.

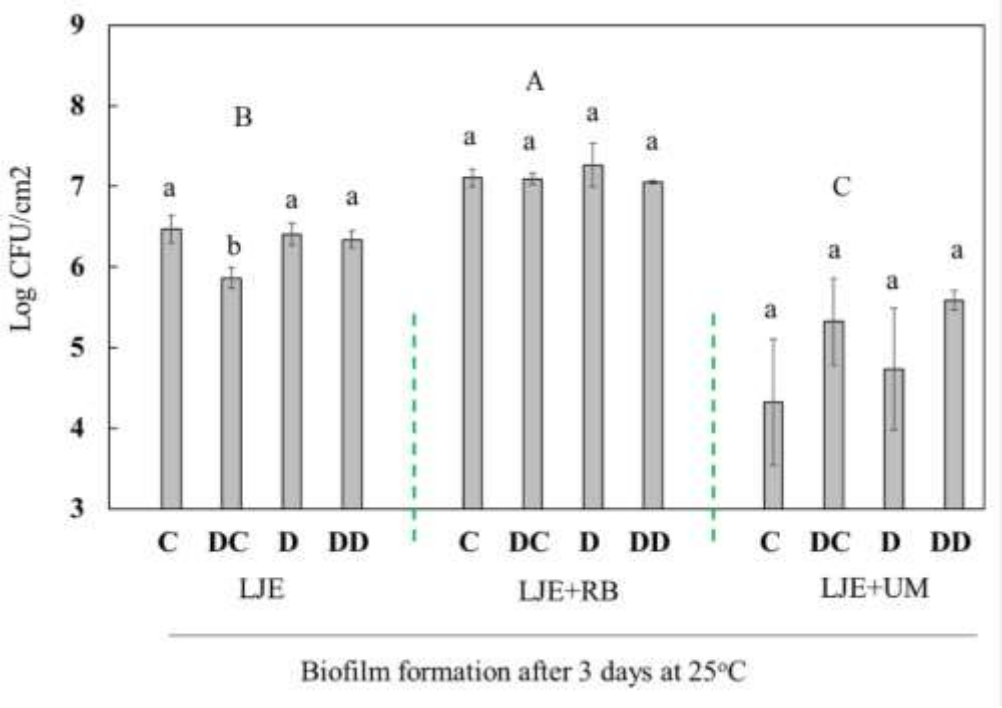

Figure 16 Non-fouling property of the top-performing FCS. **C**: SS304-B; **DC**: Dursan-coated SS304-B; **D**: SS304-Dot; **DD**: Dursan-coated SS304-Dot; **RB**, *Ralstonia insidiosa* and *Brevundimonas naejangsanensis*; **UM**, Undetermined background microflora collected from romaine lettuce leaves and environmental surface of a produce processing pilot plant. Bars denote standard deviations (n=4). Uppercase letters indicate significance levels of *Lm* biofilm populations among initial attachment and different treatment of biofilm formation. Lowercase letters indicate significance among different types of coupons tested within each treatment.

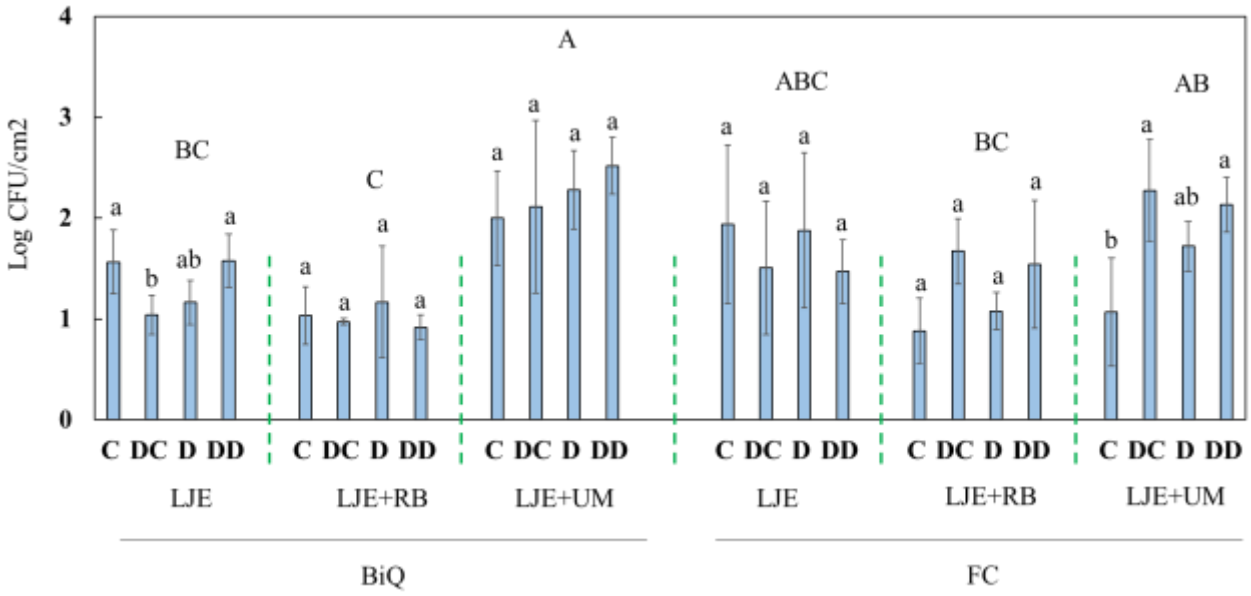

Figure 17 Reduction of *Lm* biofilms after sanitation. **C**: SS304-B; **DC**: Dursan-coated SS304-B; **D**: SS304-Dot; **DD**: Dursan-coated SS304-Dot; **BiQ**: 200 ppm quaternary ammonium compounds (BiQ, pH 6.5); **FC**:10 ppm free chlorine. Bars denote standard deviations (n=4). Uppercase letters indicate significance levels of *Lm* reduction among different sanitation treatments; lowercase letters indicate significance among different types of coupons tested within each treatment.

List of Acronyms

Acronyms	Definition
Bacterial names	
Lm	*Listeria monocytogenes*
Ec	*Escherichia coli* O157:H7
Pf	*Pseudomonas fluorescens*
Ri	*Ralstonia insidiosa*
RB	*Ralstonia insidiosa* and *Brevundimonas naejangsanensis*
UM	Undetermined background microflora
Stainless steel substrates	
SS304-B or C	Stainless steel 304 bare
SS304-4	Stainless steel 304 with #4 finishes
SS304-Dot or D	Dot-patterned stainless steel 304
SS304-Line	Line-patterned stainless steel 304
DC	Dursan-coated SS304-B
DD	Dursan-coated SS304-Dot
316L	Stainless steel 316L
Plastic substrates	
PE	Ultra-high-molecular-weight polyethylene
POM	Polyoxymethylene
PP	Polypropylene
PVC	Polyvinyl chloride
Coatings	
CrN	Chromium nitride
Ni-P-PTFE	Ni-P-polytetrafluoroethylene
TiN	Titanium nitride
Others	
BiQ	Quaternary ammonium compounds
CV	Crystal-violet

CVD	Chemical vapor deposition
DPF	Dry photoresist film
FCS	Food contact substances
FC	Free chlorine
LJE	Lettuce juice extract
NSF	(formerly) National Sanitation Foundation
PBS	Phosphate buffered saline
PTFE	Polytetrafluoroethylene
QCM-D	Quartz crystal microbalance with dissipation monitoring
SE	Standard error
TSB-YE	Tryptic soy broth–0.7% yeast extract
USP	United States Pharmacopeia Convention

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