2. הדו"ח המפורט

<u>א. דף פותח לדו"ח</u>

<u>שם התוכנית:</u> פיתוח טכנולוגיה חדשנית למניעת התבססות חיידקים על משטחי עבודה במערכות המשמשות את משק החלב.

Development of novel technology preventing a bacterial attachment and biofilm formation on surfaces related to milking equipments on dairy farms

<u>קוד הזיהוי:</u> 421-0251-15

השנה הקלנדרית עליה מוגש הדו"ח: 2015

שנת הדו"ח שלישית מתוך סה"כ 3 שנים.

<u>שמות החוקרים בקבוצות המחקר:</u>

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<u>התקציר:</u>

1 . הצגת הבעיה

היווצרות קהילות רב תאיות של חיידקים המכונות ביופילמים במערכות המשמשות את משק החלב מקנה לחיידקים עמידות מוגברת בפני טיפולים אנטימיקרוביאליים ולכן מהווה בעיה קשה בתחום של איכות ובטיחות של חלב ומוצריו.

2 . מטרות המחקר

פיתוח משטחים חדשניים המונעים יצירת הביופילם והתבססות חיידקים במערכות המשמשות את משק החלב ע"י שיבוש תהליכי ההיצמדות ויצירת הביופילם.

3 . שיטות העבודה

ציפוי משטחים נעשה באמצעות נידוף תרמי של פחמימנים בעלי תכונות סופר-הידרופוביות. המשטחים אופיינו ע"י מיקרוסקופיה קונפוקלית, אלקטרונית ודיפרקציית קרני איקס. היעילות למניעת יצירת הביופילם אובחנה ע"י אפיון מורפולוגי באמצעות צביעה פלואורסנטית ואנליזת מיקרוסקופיה קונפוקלית. כמוכן, על מנת לאשש את התוצאות נעשה שימוש בשיטת ספירה חייה של חיידקי הביופילם שהתפתחו על משטחים שצופו בשעוות שונות על מצעי פלב"מ, זכוכית ופוליסטירן.

4 . תוצאות עיקריות

פותחה טכנולוגיה חדשנית המבוססת על ציפוי משטחי זכוכית, פוליסטירן ופלב"מ ע"י נידוף תרמי של השעוות הבאות: $C_{24}F_{50}$, $C_{36}H_{74}$, $C_{50}H_{102}$, $C_{36}H_{74}+C_{50}H_{10}$. כל אחת מהשעוות יוצרת מבנה תלת מימדי עם גדלי גבישים שונים, כאשר המבנים $C_{24}F_{50}$ ו $C_{36}H_{74}+C_{50}H_{102}$ הם מבנים היררכיים. ערכי זוויות המגע (מים) על משטחים היררכיים הינם גבוהים ביותר בעוד שערכי ההיסטרזיס הינם נמוכים ביותר. לפי אנליזת מיקרוסקופיה קונפוקלית נמצא כי היכולת לבנות ביופילם על ידי חיידקי הבצילוס נפגעה מאוד בעקבות ציפוי משטחי זכוכית ופלב"מ בשעוות: $C_{24}F_{50}$ או $C_{36}H_{74}+C_{50}H_{102}$; ואילו היווצרות הביופילם ע"י חיידקי הפסאודומונס נפגעה בצורה קשה בציפוי ע"י $C_{36}H_{74}+C_{50}H_{102}$. התוצאות הללו מצביעות על כך שהמשטח הדו-שכבתי מספק פגיעה המשמעותי ביותר להיווצרות הביופילם על ידי חיידקים גרם חיוביים וגרם שליליים. בהמשך, מצאנו כי המשטחים שפותחו מראים פגיעה משמעותית (עד 99.9% עיכוב) גם בשלבי הבשלת הביופילם הן ע"י חיידקי הבצילוס והן ע"י חיידקי הפסאודומונס, במיוחד בציפוי ע"י $C_{36}H_{74}+C_{50}H_{102}$.

5 . מסקנות והמלצות לגבי יישום התוצאות.

הצלחנו לפתח טכנולוגית ציפוי משטחים הרלוונטיים לתעשיית החלב. משטחים המצופים ב-$C_{36}H_{74}+C_{50}H_{102}$ מראים תוצאות מבטיחות במניעת יצירת הביופילם ע"י חיידקים שונים שנבדקו בעבודת מחקר זו.

מעריכים מומלצים לבדיקת הדוח המדעי

1. פרופ' שלמה סלע (מינהל המחקר החקלאי)
2. פרופ' דורון שטיינברג (האוניברסיטה העברית)
3. ד"ר מיטל רכס (האוניברסיטה העברית)

הצהרת החוקר הראשי:

הממצאים בדו"ח זה הינם תוצאות ניסויים.

הניסויים מהווים המלצות לחקלאים: כן */ לא (מחק את המיותר)

*** במידה וכן , על החוקר להמציא פרטים על הגוף שבאמצעותו מופץ הידע (כמו: שה"ם)**

חתימת החוק  **תאריך :** 15.3.16

רשימת פרסומים שנבעו מהמחקר:

Pechook, S., Sudakov, K., Polishchuk, I., Ostrov, I., Zakin, V., Pokroy, B. and Shemesh, M. Bioinspired passive anti-biofouling surfaces preventing biofilm formation. *Journal of Materials Chemistry B.* 2015, 3, 1371-1378.

תוכן העניינים

עמוד

ב. גוף דו"ח המחקר

<u>מבוא</u>

Microbial damages caused by bacteria in the dairy industry are a fundamental threat to the safety and quality of milk products. Many bacteria in industrial settings tend to form multicellular communities known as biofilms (L. Hall-Stoodley, J. W. Costerton, & P. Stoodley, 2004; Kolter & Greenberg, 2006). Individual cells in the biofilms are deeply embedded and protected by a self-produced matrix that consists mainly of sugars and proteins, which form a physical barrier (L. Hall-Stoodley, et al., 2004; Stewart & Costerton, 2001) (Shemesh, Kolter, & Losick, 2010). Biofilms represent one of the most successful strategies for bacteria to survive unfavorable environmental conditions, for instance in the food industry. In order to ensure the safety and quality of dairy food, there is a fundamental requirement of effective cleaning and sanitizing procedures. Otherwise, residual spores and bacteria on inadequately cleaned surfaces can quickly form multicellular biofilms that are extremely difficult to remove. Biofilms are not only a potential source of contamination, but can also increase corrosion rate, reduce heat transfer and increase fluid frictional resistance. Therefore, mitigation of biofilm forming species will enable the development of novel means and technologies for preventing biofilm formation and subsequent contamination of dairy products.

Biofilms are problematic in a broad range of areas, particularly in the food, environmental and biomedical fields (Simoes, Simoes, & Vieira, 2010). A considerable problem in the food industry is the formation of biofilms in dairy processing plants, particularly those formed by members of the *Bacillus* genus (Sharma & Anand, 2002; Simoes, et al., 2010). *B. cereus* is a spore-forming pathogenic bacterium that causes two distinct types of food poisoning, the diarrheal and emetic syndromes, as well as a variety of local and systemic infections (Kotiranta, Lounatmaa, & Haapasalo, 2000). It has been shown to be capable of forming biofilms on stainless steel, plastic and glass wool (Auger, Krin, Aymerich, & Gohar, 2006; Oosthuizen, et al., 2002; Peng, Tsai, & Chou, 2001, 2002).

Pseudomonas spp., considered to be one of the most important groups of bacteria in clinical as well as in industrial settings (Simoes, et al., 2010; Sydnor & Perl, 2011). Biofilms of *Bacillus* and *Pseudomonas* species are thus jointly

regarded as the most significant microbiological problem in the food industry, because the damage they inflict on the quality and safety of food products may impact public health as well as the economy (Simoes, et al., 2010). Their ubiquitous nature, combined with their ability to grow even at refrigerator temperatures, make them difficult to control.

Prevention of biofilm formation would clearly be a much more desirable option than treating it, and a wide range of bacteria-resistant surfaces has been proposed for this purpose. Examples are surfaces modified with nanoparticles (ZnO, TiO2 and carbon nanotubes) that mechanically damage the bacterial cells (Banerjee, Pangule, & Kane, 2011). However, most fabrication methods rely on one of two main strategies. The first is based on the release of biocidal compounds such as silver or copper ions, various antibiotics, chlorohexidine, or quaternary ammonium salts (Banerjee, et al., 2011; Meyer, 2003; Zhao, Chu, Zhang, & Wu, 2009). The second strategy depends on inhibition of adhesion, and various methods have been suggested for this purpose: using hydrophilic surfaces to create fully hydrated surfaces, the best-known example being polyethylene glycol (PEG) polymers (Banerjee, et al., 2011; Harris, 1992; Park, et al., 1998). Other methods in this category make use of zwitterionic materials (Jiang & Cao, 2010), novel liquid-infused structured surfaces (Epstein, Wong, Belisle, Boggs, & Aizenberg, 2012), amphiphilic block copolymer surfaces (Krishnan, Weinman, & Ober, 2008), and smart stimuli-responsive materials designed as fouling-release surfaces (Banerjee, et al., 2011). Both strategies, however, have drawbacks. Biocidal-releasing compounds usually have only short-term efficiency because of the limited amount of the biocidal compound or the resistance to the compound developed by the bacteria (Luanne Hall-Stoodley, J. William Costerton, & Paul Stoodley, 2004; Stewart & Costerton, 2001). PEG coatings undergo oxidation damage that leads to loss of efficiency (Gaberc-Porekar, Zore, Podobnik, & Menart, 2008). Metallic nanoparticles might have harmful effects on human tissue (Marambio-Jones & Hoek, 2010). Therefore, in most industrial and medical applications the main strategy for preventing biofilm formation currently still relies on regular and aggressive cleaning and disinfection of bacterial contact surfaces (Simoes, et al., 2010).

Previous reports have described the antifouling properties of natural surfaces such as taro leaves (Ma, Sun, Gleichauf, Lou, & Li, 2011) and cicada wings

(Hasan, et al., 2012) and of bioinspired surfaces such as slippery surfaces inspired by the Nepenthes pitcher plant (Epstein, et al., 2012). In addition, several superhydrophobic surfaces have demonstrated antibacterial properties (Berendjchi, Khajavi, & Yazdanshenas, 2011; Chapman & Regan, 2012; Chung, et al., 2012).

<u>מטרות המחקר</u>

מטרת המחקר היא לפתח משטחים חדשניים אשר ימנעו יצירת הביופילם והתבססות חיידקים במערכות המשמשות את משק החלב. המחקר המוצע מתבסס על ההנחה כי שיבוש תהליך היצמדות ויצירת הביופילם ימנע התבססות חיידקים ונבגים על משטחים במשק החלב.

<u>פירוט עיקרי הניסויים שבוצעו וכלל התוצאות שהתקבלו לתקופת הדו"ח</u>

Development of surface preventing bacterial attachment

In order to develop novel surfaces preventing bacterial attachment and biofilm formation, we generated new biomimetic superhydrophobic surfaces that are formed via the self-assembly of paraffin or fluorinated wax crystals. Surfaces were prepared by thermal deposition of n-paraffin and fluorinated waxes on glass, polystyrene and steel substrates using a Moorfield MiniLab evaporator. The waxes hexatriacontane ($C_{36}H_{74}$, 98%), pentacontane ($C_{50}H_{102}$, ≥97%) and perfluorotetracosane ($C_{24}F_{50}$) were purchased from Sigma-Aldrich (France). The deposition procedure was conducted in a vacuum chamber at 5×10^{-6} mbar. Samples were positioned on a holder 12 cm above a crucible loaded with 40−50 mg of an n-paraffin wax or 110 mg of perfluorotetracosane. The system was slowly heated from 70 °C to 120 °C. Evaporation occurred at 120 ± 5 °C within 10-15 min. Evaporated specimens were transferred to room temperature (25 °C). Hierarchical structures were prepared by thermal evaporation of a $C_{50}H_{102}$ wax layer on top of a previously evaporated $C_{36}H_{74}$ wax. Surface wetting properties were assessed by measuring contact angles with an Attension Theta tensiometer. Measurements were performed with 7 µL of high purity water (milli Q). Contact angle hysteresis was measured with alternating drops of 7 ± 13 µL. Surface imaging was performed by high-resolution scanning electron microscopy (Zeiss Ultra Plus HR-SEM). Roughness was assessed by confocal microscopy (Leica DCM 3D). We examined several waxes (both paraffin and

fluorinated). Each substrate was separately coated with each of the following waxes: $C_{24}F_{50}$, $C_{36}H_{74}$, $C_{50}H_{102}$, and $C_{36}H_{74}$+$C_{50}H_{102}$. Each wax formed a 3D crystalline structure with crystals of different sizes (as a function of the molecular weight), where both $C_{24}F_{50}$ and $C_{36}H_{74}$+$C_{50}H_{102}$ formed two-tiered hierarchical crystalline structures (Table 1 and Figure 1).

Table 1: crystal size as measured via HRSEM and confocal microscopy

surface	Length [µm]	Width	Length 2nd	Width 2nd	RMS roughness
$C_{36}H_{74}$	1-1.5	0.1 µm	-	-	0.055 µm
$C_{50}H_{102}$	0.2-0.3	50nm	-	-	1.9 nm
$C_{36}H_{74}$+$C_{50}H_{102}$	1-1.5	0.1 µm	0.2-0.3 µm	50nm	0.057 µm
$C_{24}F_{50}$	1	100nm	0.2-0.5 µm	20-50nm	0.04 µm

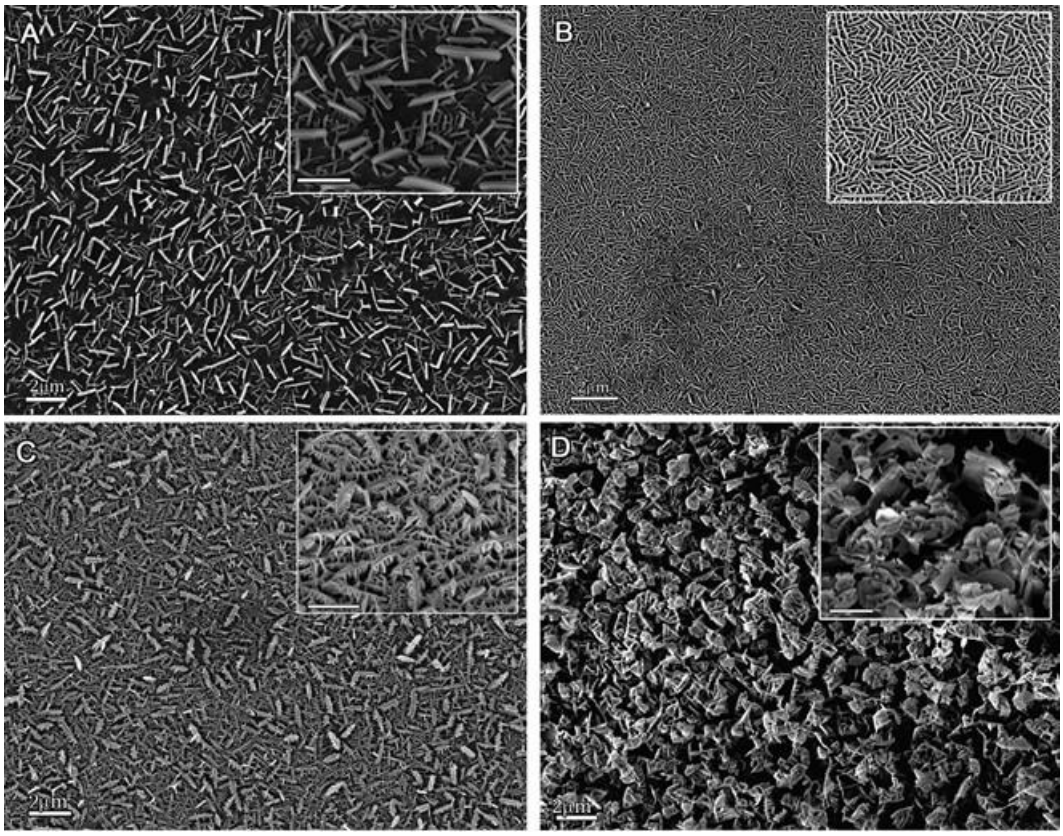

Figure 1. HR-SEM micrographs of 3D crystalline structures thermally evaporated on glass substrates (A) $C_{36}H_{74}$, (B) $C_{36}H_{74}$+ $C_{50}H_{102}$, (C) $C_{50}H_{102}$, and (D) $C_{24}F_{50}$. Insets were acquired at a 30° tilt angle. Scale bar is 1µm.

All examined surfaces demonstrated superhydrophobic behaviour (contact angel >150° contact angel hysteresis lower than 10°), the lowest contact angel was observed for $C_{50}H_{102}$ surfaces which exhibited the lowest surfaces roughness. The hierarchical surfaces demonstrated, in addition to extremely high water contact angles (C.A >170°) and low contact-angle hysteresis (Table 2), an ability to support water droplets of ~100 pl (Figure 2) indicating elevated stability of the superhydrophobic wetting state (Cassie wetting state(Cassie & Baxter, 1944)) (Michael & Bhushan, 2007).

Table 2: water contact angel and contact angel hysteresis for various surfaces

surface	Water Contact angel (˚)	Water Contact angel hysteresis (˚)	EG Contact angel (˚)
$C_{36}H_{74}$	170	3	121
$C_{50}H_{102}$	151	8	103
$C_{36}H_{74}$+$C_{50}H_{102}$	171	2	149
$C_{24}F_{50}$	172	2	165

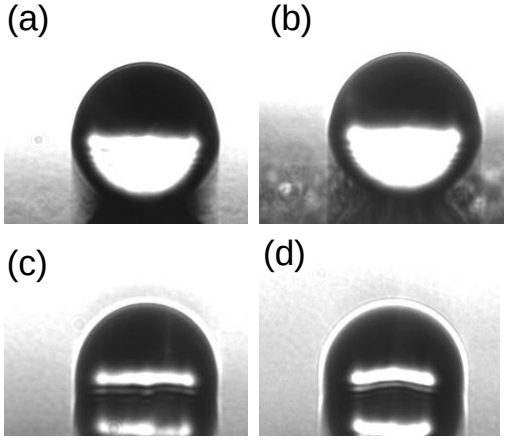

Figure 2**.** ~100 pl water droplets on: a) $C_{36}H_{74}$+ $C_{50}H_{102}$, b) $C_{24}F_{50}$, c) $C_{36}H_{74}$ and d) $C_{50}H_{102}$.

Determining the effect of created surfaces on biofilm formation

To examine the effects of surface modification with hydrophobic wax on biofilm formation we analyzed two pathogenic bacteria, *B. cereus* (Gram positive) and

P. aeruginosa (Gram negative), for their ability to form biofilms on stainless steel, glass and polystyrene. The strains were propagated in Lysogeny broth (LB; 10 g tryptone, 5 g yeast extract, 5 g NaCl per liter) or on solid LB medium supplemented with 1.5% agar. For biofilm generation, bacteria were grown to the stationary phase in LB liquid medium in shaking culture. The generated cultures were seeded (1:100 dilution) into sterile polystyrene multidishes containing different substrates and were inoculated statically (without agitation) into fresh media. To visualize the constructed biofilms the substrates were removed from the wells, washed with PBS buffer, and stained by using a FilmTracerTM LIVE/DEAD Biofilm Viability Kit (Molecular Probes) according to the manufacturer's protocol. Stained samples were visualized under an Olympus IX81 confocal laser scanning microscope (CLSM, Japan). Live cells were stained green and dead cells were stained red. Fluorescence emission of the stained samples was measured using an Olympus IX81 CLSM equipped with 488-nm argon-ion and 543-nm helium-neon lasers.

Confocal scanning laser microscopy (CSLM) images showed that the cells of *B. cereus* could not form biofilm (in comparison to their adhesion to control uncoated surface) after 24 hours of incubation on steel, glass or polystyrene substrates coated with either $C_{24}F_{50}$ or $C_{36}H_{74}$+$C_{50}H_{102}$, while only slight inhibition was observed for $C_{36}H_{74}$ surfaces (Figure 3). Over the same time span, biofilm formation by *P. aeruginosa* on substrates coated with $C_{36}H_{74}$+$C_{50}H_{102}$ was strongly prevented. However, other tested coatings, such as $C_{24}F_{50}$ and $C_{50}H_{102}$, did not significantly inhibit the formation of *P. aeruginosa* biofilms (Figure 4).

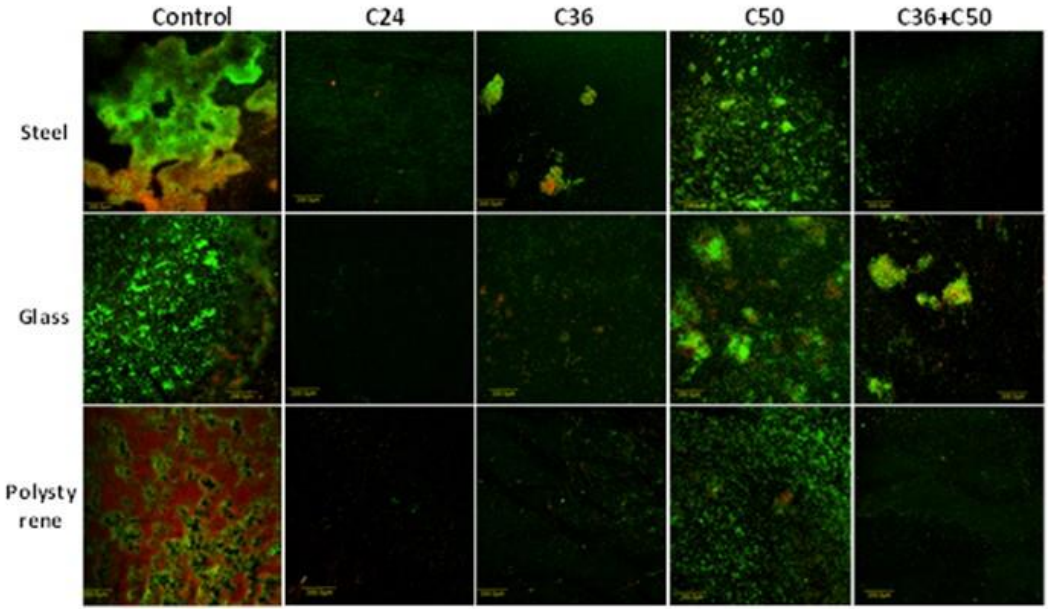

Figure 3. CSLM images of *B. cereus* ATCC 10987 biofilms generated on steel, glass and polystyrene substrates: uncoated control, coated with C24, C36, C50 and C36C50. *B. cereus* Biofilms were grown on surfaces for 24 h and stained with SYTO9 and PI of FilmTracer™ LIVE/DEAD Biofilm Viability Kit (Molecular Probes, OR). Fluorescence emission of the stained samples was measured using an Olympus IX81 Confocal laser scanning microscope (Japan).

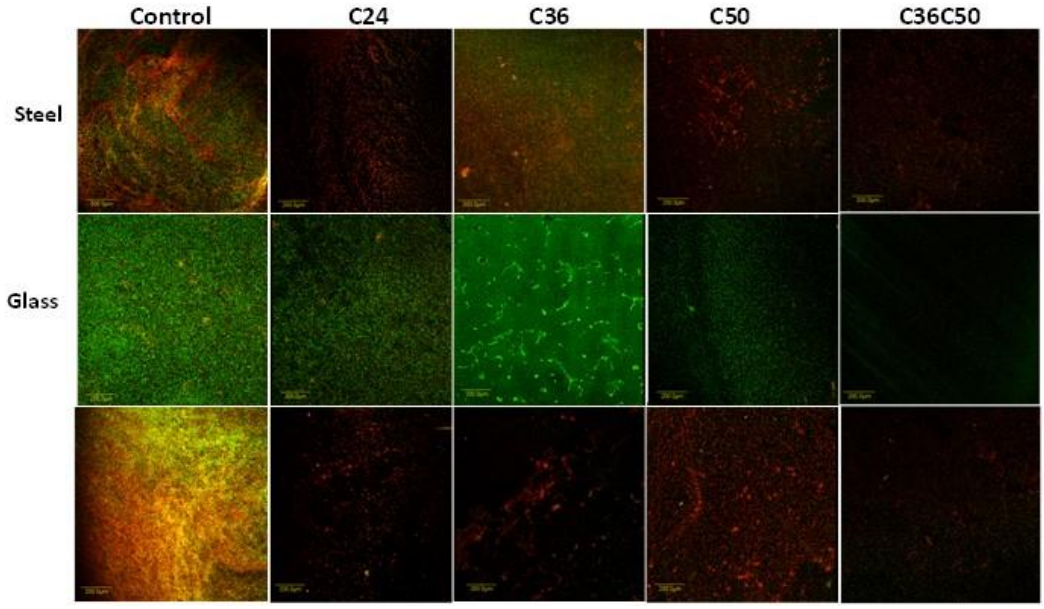

Figure 4. CSLM images of *P. aeruginosa* PA14 biofilms generated on steel, glass and polystyrene substrates: uncoated control, coated with C24, C36, C50 and C36C50. *P. aeruginosa* Biofilms were grown on coated surfaces for 24 h and stained with SYTO9 and PI of FilmTracer™ LIVE/DEAD Biofilm Viability Kit (Molecular Probes, OR). Fluorescence emission of the stained samples was measured using an Olympus IX81 Confocal laser scanning microscope (Japan).

To further support our results we used the plating method to quantify colony-forming units (CFU) of viable bacteria adhering to various wax surfaces formed on different substrates. Compared to uncoated control surfaces, reductions of 80% to 99.4% were observed in the numbers of *B. cereus* cells adhering to wax (either paraffin or fluorinated) surfaces formed on glass, stainless steel or polystyrene substrates (Figure 5A). Of all the surfaces examined, $C_{24}F_{50}$ and $C_{36}H_{74}+C_{50}H_{102}$ consistently demonstrated the most significant reductions in bacterial adhesion. The adhesion of *B. cereus* to glass, polystyrene and steel substrates coated with $C_{24}F_{50}$ was reduced by 98%, 97%, and 82%, respectively. Notably, all substrates coated with $C_{36}H_{74}+C_{50}H_{102}$ showed significant reductions (>97%) in *B. cereus* adhesion and biofilm formation. On examining the adhesion of *P. aeruginosa* to the wax surfaces, we observed reductions of 42% to 90% in the numbers of adherent cells compared to control surfaces (Figure 5B). Of all the examined surfaces, the $C_{36}H_{74}+C_{50}H_{102}$ hierarchical structure exhibited the largest reductions in adhesion of *P. aeruginosa* to glass, steel and polystyrene substrates (by 87%, 89% and 90%, respectively). The $C_{24}F_{50}$ hierarchical structure also effectively reduced *P. aeruginosa* adhesion and biofilm formation (Figure 5B). These results strongly suggest that the two-tiered paraffin $C_{36}H_{74}+C_{50}H_{102}$ surface provided the most effective reduction of both Gram-positive and Gram-negative bacteria adhesion and hence of biofilm formation on the different types of substrates.

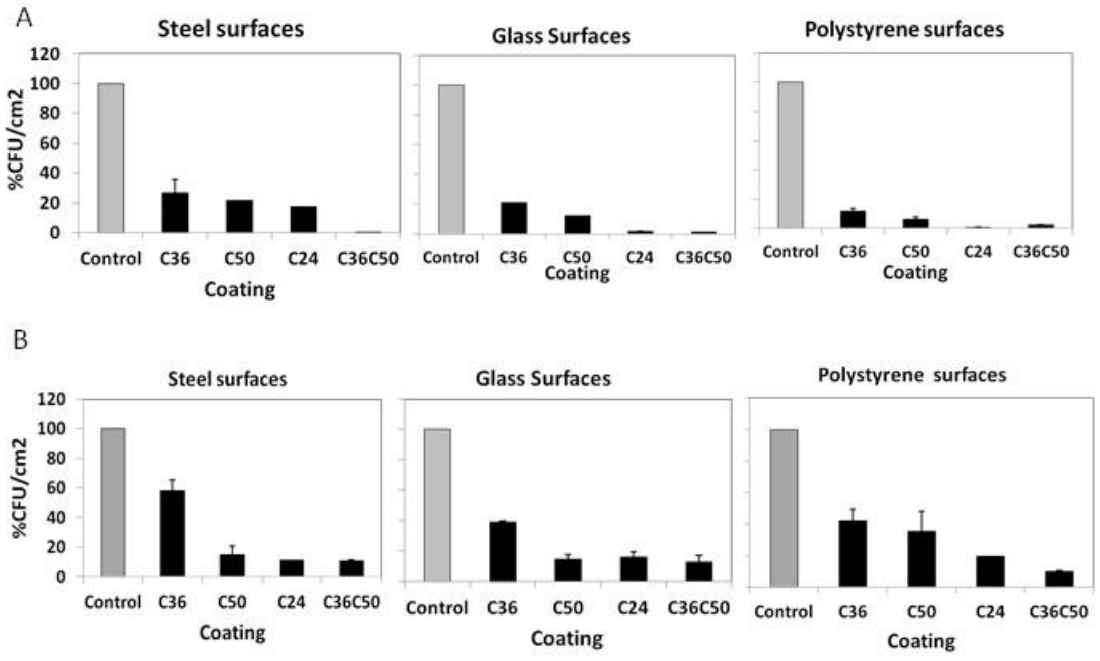

Figure 5. Live bacterial counts on uncoated steel, glass or polystyrene surfaces (control) and on surfaces coated with $C_{24}F_{50}$, $C_{36}H_{74}$, $C_{50}H_{102}$ or $C_{36}H_{74}$+$C_{50}H_{102}$. Biofilms were grown in LB liquid media for 24 h (A) *B. cereus* biofilms, (B) *P. aeruginosa* biofilms.

We further examined the inhibitory capacity of the developed surfaces during long-term biofilm formation by *B. cereus* and by *P. aeruginosa*. All wax surfaces formed on steel substrate demonstrated strong inhibition of biofilm formation throughout the 7 days of incubation with each species (97.6-99.9% inhibition of *B. cereus* and 97.8-99.9% inhibition of P. aeruginosa, Figure 6). At the end of this incubation period there was impressive inhibition (almost 3-log) in P. aeruginosa biofilm formation by the C36H74+C50H102 structured surface (99.9%) and significant inhibition by both C36H74+C50H102 and C24F50 hierarchical structures of *B. cereus* biofilm formation (99.8% and 99.9% respectively). A fascinating finding was that the inhibition of mature (7-day) biofilm formation was more marked than that of young (24-hour) biofilm. This phenomenon might be explained by possible damage caused by waxed surfaces to the biofilm maturation process (a critical step during biofilm formation), with consequent inability of the bacteria to aggregate to form a confluent and mature biofilm, even if some initial adhesion (as seen after 24 h of incubation) has occurred.

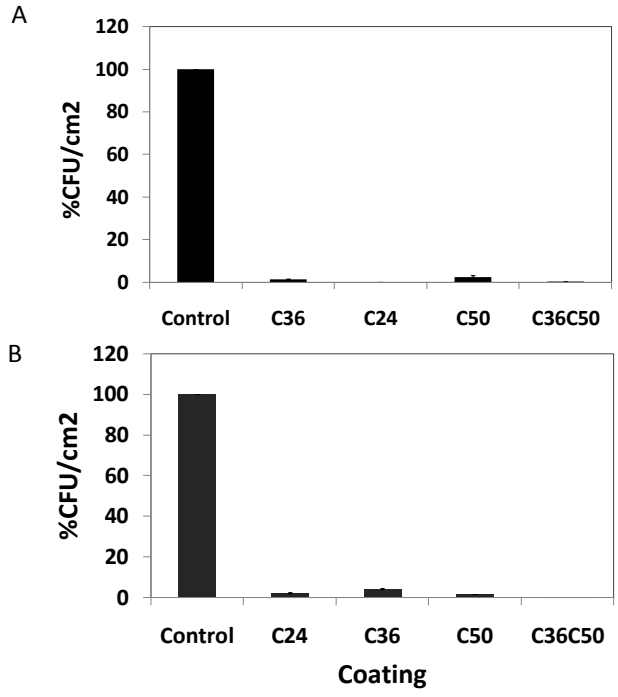

Figure 6. Live bacterial count on a steel substrate. A stainless steel substrate was left

uncoated (control) or was coated with C24F50, C36H74, C50H102, or C36H74+C50H102. Biofilms were grown in LB liquid media for 7 days at 30 °C for (A) *B. cereus* or (B) *P. aeruginosa*.

In addition, we also examine the effect of the generated surfaces on the ability of newly identified *B. licheniformis* S127 strain (Ostrov, et al., 2015) to form biofilms on modified stainless steel, glass and polystyrene. CLSM images show that the cells of *B. licheniformis* S127 (in comparison to their adhesion to control (uncoated) surfaces) could not adhere successfully to form confluent biofilms on stainless steel, glass or polystyrene surfaces coated with polymers designated as C24F50 and C36H74+C50H102 (Figure 7).

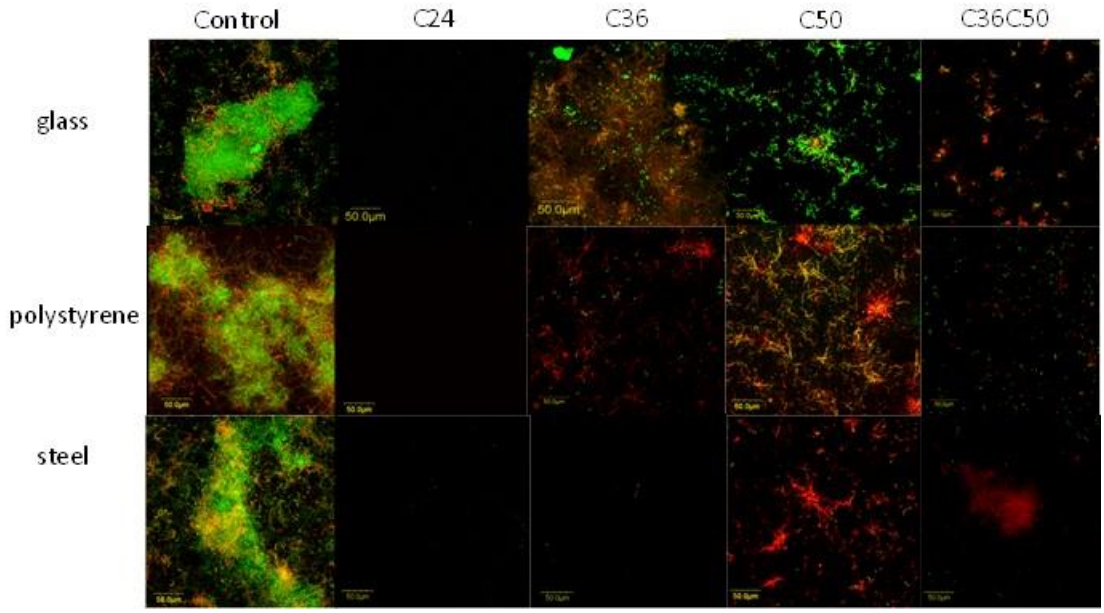

Figure 7. CSLM images of *B. licheniformis* S127 biofilms generated on C24F50, C36H74, C50H102 and C36H74+C50H102 wax surfaces thermally evaporated on steel, glass and polystyrene substrates after 48 h of cultivation in batch culture.

Consequently, the C24F50 and C36H74+C50H102 coatings were chosen for further investigations, as they have shown the highest inhibition of biofilm formation on all studied types of surfaces. To further support our results, we used the plating method to quantify colony-forming units (CFU) of viable bacteria adhering to C24F50 and C36H74+C50H102 polymer surfaces generated on glass, stainless steel or polystyrene. Both coatings demonstrated significant reductions in bacterial adhesion compared to uncoated control, providing effective inhibition of biofilm formation (Figure 8).

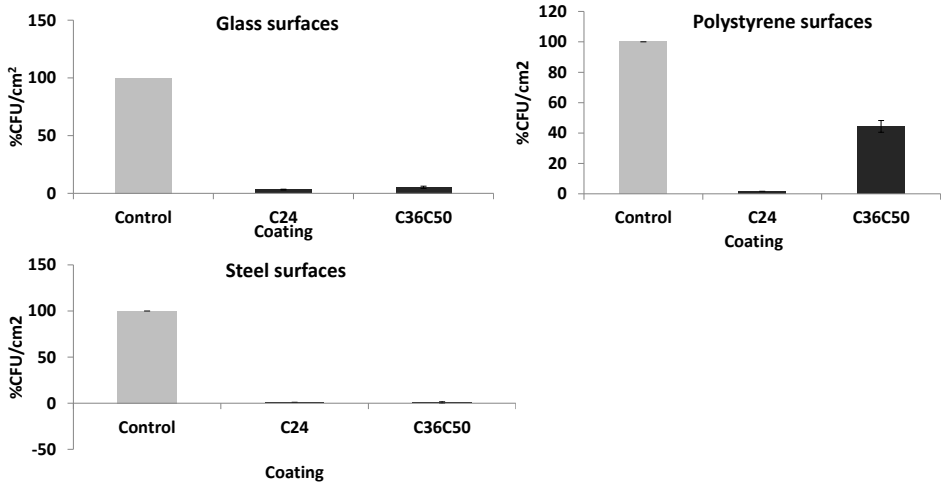

Figure 8. Live bacterial counts of *Bacillus licheniformis* S127 on uncoated glass, steel and polystyrene substrates (control) and on substrates coated with C24F50, or C36H74+C50H102 after 48 h of cultivation.

We also examined the inhibitory capacity of C24F50 and C36H74+C50H102 surfaces during long term biofilm formation of *B. licheniformis*. CLSM (Figure 9) and CFU (Figure 10) quantitation has shown that the developed coatings demonstrated strong inhibition of biofilm formation throughout the 7 days of incubation with bacteria.

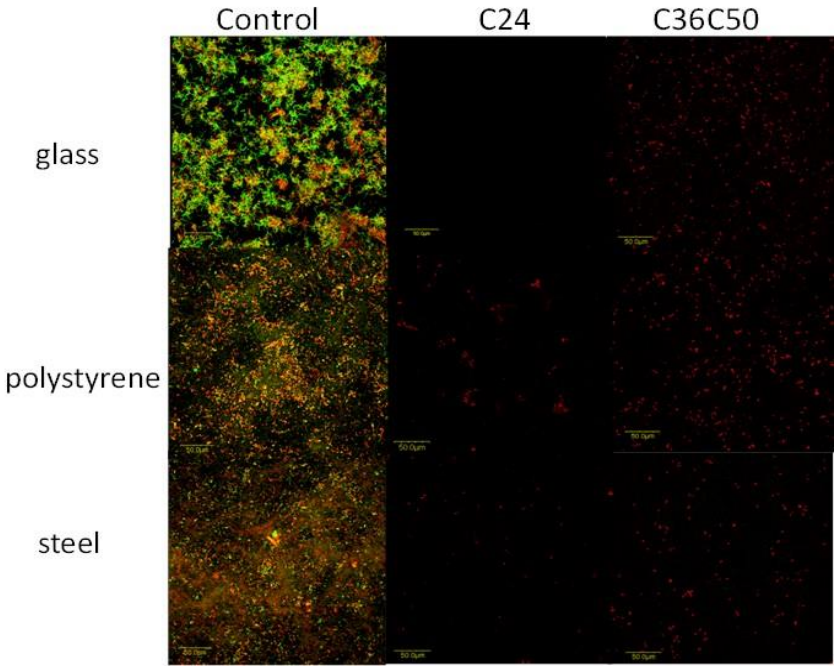

Figure 9. CSLM images of *B. licheniformis* S127 biofilms generated on $C_{24}F_{50}$ and $C_{36}H_{74}$+$C_{50}H_{102}$ wax surfaces thermally evaporated on steel, glass and polystyrene substrates following 7 days of cultivation in batch culture.

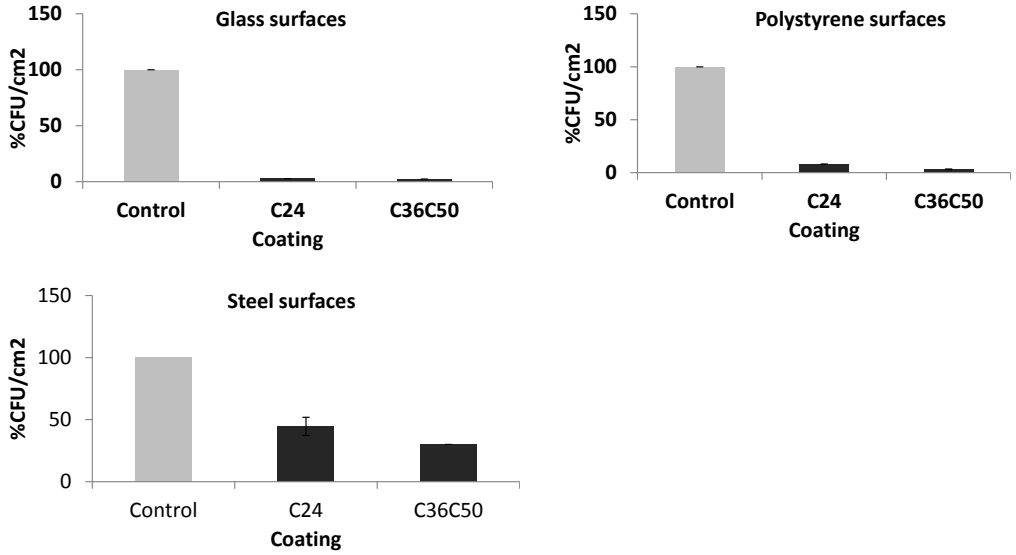

Figure 10. Live bacterial counts of *B. licheniformis* S127 on uncoated glass, steel and polystyrene substrates (control) and on substrates coated with $C_{24}F_{50}$, or $C_{36}H_{74}$+$C_{50}H_{102}$ after 7 d of cultivation.

דיון

It appears that most bacteria in their natural state exist as matrix-enclosed, surface-associated biofilms. In this mode of growth, the bacteria are largely protected from environmental insults as well as from various antimicrobial treatments. Since bacterial biofilms, once formed, are extremely resistant to antimicrobial treatments, the results of this study are of vast importance in the field of microbiology, as the antimicrobial coatings tested here can be used to modify any industrial or clinical surface to prevent bacterial colonization and biofilm formation.

Our approach incorporates an easily applicable coating technology based on thermal evaporation of waxes (paraffin or fluorinated), allowing the formation of superhydrophobic surfaces that prevent biofilm formation on stainless steel, polystyrene or glass substrates. These 3D crystalline wax surfaces form a Cassie wetting state (heterogeneous surface that combines wax and air pockets), reducing the contact area between a bacterium and the surface and thereby interrupting bacterial adhesion, thus preventing the initial step of biofilm formation. Our aim was to devise an approach that could be applied in substrates used in food and other industries for the development of novel surfaces that would prevent the adhesion of bacteria and consequently reduce

biofilm formation. To test the efficacy of the generated surfaces, we examined the interactions of bacteria with wax-coated stainless steel, glass, and polystyrene substrates.

As shown in Figures 3 and 4, biofilm formation by *B. cereus* on $C_{24}F_{50}$-coated and $C_{36}H_{74}$+$C_{50}H_{102}$-coated surfaces of all examined substrates was significantly reduced. In the case of *P. aeruginosa*, notable reductions in biofilm formation were seen on $C_{24}F_{50}$, $C_{50}H_{102}$ and $C_{36}H_{74}$+$C_{50}H_{102}$ surfaces (Figures 3 and 4). A possible explanation might derive from differences in crystal size, density, and surface roughness. $C_{24}F_{50}$ and $C_{36}H_{74}$+$C_{50}H_{102}$ are hierarchical structures with high surface roughness. Lower surface roughness results in reduced hydrophobicity (that is, lower contact angles and higher contact-angle hysteresis) and consequently reduced water repulsion. Furthermore, hierarchical structures are known for their higher stability of the Cassie wetting state (Michael & Bhushan, 2007; Nosonovsky & Bhushan, 2007), which might be beneficial for long-term antifouling capabilities.

The difference observed in the adhesion of *Bacillus* and *Pseudomonas* to surfaces can be attributed to the differences in the adherence mechanisms of Gram-negative (*Pseudomonas*) and Gram-positive (*Bacillus*) bacteria. Studies have shown that adhesion and biofilm formation by *Bacillus* species depend on the creation of a conditioning film that adsorbs to the surface (Jain & Bhosle, 2009; Vilain, Pretorius, Theron, & Brozel, 2009). In *Pseudomonas* species, on the other hand, adherence and biofilm formation occur through direct bacteria-surface interactions in the absence of conditioning film (Giltner, et al., 2006). As seen in Figures 3 and 4, on most coated surfaces the cells of *B. cereus* formed poor biofilms compared to the biofilms of *P. aeruginosa*.

The exceptional ability of the $C_{36}H_{74}$+$C_{50}H_{102}$ and $C_{24}F_{50}$ hierarchical structures to resist attachment and biofilm formation of the tested bacteria independently of any specific chemical or physical feature of the cells points to the potential feasibility of using this coating as a general antifouling material for resisting biofilms formed by a broad spectrum of bacteria. Given our experimental observations, it is conceivable that the bacteria have difficulty in developing a confluent biofilm efficiently since they are not able to be in contact with substrates coated with $C_{36}H_{74}$+$C_{50}H_{102}$ and $C_{24}F_{50}$. Importantly, the $C_{36}H_{74}$+$C_{50}H_{102}$ and $C_{24}F_{50}$ wax coatings were nontoxic to the tested bacteria;

thus, the action of the $C_{36}H_{74}+C_{50}H_{102}$/ $C_{24}F_{50}$-coated surfaces is most probably attributable to biofilm formation. This is an important requirement for suppression of bacterial resistance to the antimicrobial treatment.

In summary, this work has demonstrated that our modified surfaces almost completely prevent the formation of biofilms by Gram positive as well as Gram negative problematic bacteria in industrial settings. Importantly, the wax surfaces used in this study can be formed on a great variety of materials and intricately shaped surfaces, making the technology potentially feasible for various medical and industrial applications.

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3. ***סיכום עם שאלות מנחות***

נא להתייחס לכל השאלות בקצרה ולעניין, ב-3 עד 4 שורות לכל שאלה (לא תובא בחשבון חריגה מגבולות המסגרת המודפסת).

שיתוף הפעולה שלך יסייע לתהליך ההערכה של תוצאות המחקר.

הערה: נא לציין הפנייה לדו"ח אם נכללו בו נקודות נוספות לאלה שבסיכום.

מטרות המחקר תוך התייחסות לתוכנית העבודה.
מטרת המחקר היא לפתח משטחים חדשניים אשר ימנעו יצירת הביופילם והתבססות חיידקים במערכות המשמשות את משק החלב. המחקר המוצע מתבסס על ההנחה כי שיבוש תהליך היצמדות ויצירת הביופילם ימנע התבססות חיידקים על משטחים במשק החלב.
עיקרי התוצאות.
פותחה טכנולוגיה חדשנית המבוססת על ציפוי משטחי זכוכית ופלדה בלתי מחלידה על ידי נידוף תרמי של פחמימנים ארוכי-שרשרת בעלי תכונות סופר-הידרופוביות. להכנת הציפויים נעשה שימוש בשעוות הבאות: $C_{24}F_{50}$, $C_{36}H_{74}$, $C_{50}H_{102}$, $C_{36}H_{74}+C_{50}H_{10}$. המשטחים אשר התקבלו מציפוי בשעוות אלו הינם בעלי טקסטורה גבישית וזווית מגע גבוהה מאוד. כמוכן, נמצא כי היכולת של חיידקי הבצילוס (גרם חיובי) לבנות ביופילם נפגעה מאוד בעקבות ציפוי המשטחים בשעוות: $C_{24}F_{50}$ או $C_{36}H_{74}+C_{50}H_{102}$; ואילו היווצרות הביופילם ע"י חיידקי הפסאודומונס (גרם שלילי) נפגעה בצורה קשה בציפוי ע"י $C_{36}H_{74}+C_{50}H_{102}$. בנוסף, משטחים אלו פגעו באופן משמעותי גם בהבשלת הביופילם המהווה שלב חשוב להתפתחות הביופילם.
מסקנות מדעיות וההשלכות לגבי יישום המחקר והמשכו. האם הושגו מטרות המחקר לתקופת הדו"ח?
הממצאים מצביעים כי הצלחנו לפתח טכנולוגית ציפוי משטחים הרלוונטיים לתעשיית החלב. כמוכן, משטחים אשר צופו ב- $C_{36}H_{74}+C_{50}H_{102}$ מראים תוצאות מבטיחות מאוד במניעה ועיכוב היווצרות הביופילם ע"י חיידקים שנבדקו עד כה. לפיכך, מטרות המחקר אכן הושגו בעבודה זו.
בעיות שנותרו לפתרון ו/או שינויים (טכנולוגיים, שיווקיים ואחרים) שחלו במהלך העבודה; התייחסות המשך המחקר
הפצת הידע שנוצר בתקופת הדו"ח: **פרסומים בכתב** - ציטט ביבליוגרפי כמקובל בפרסום מאמר מדעי;
מאמר מדעי אשר מתאר את ממצאי המחקר סוכם ופורסם בכתב עת בינלאומי כדלקמן: Pechook, S., Sudakov, K., Polishchuk, I., Ostrov, I., Zakin, V., Pokroy, B. and Shemesh, M. Bioinspired passive anti-biofouling surfaces preventing biofilm formation. *Journal of Materials Chemistry B.* 2015, 3, 1371-1378.
פרסום הדו"ח: אני ממליץ לפרסם את הדו"ח: (סמן אחת מהאופציות)
➢ ללא הגבלה (בספריות ובאינטרנט)
➢ חסוי – לא לפרסם
האם בכוונתך להגיש תוכנית המשך בתום תקופת המחקר הנוכחי? כן* - לא -

*יש לענות על שאלה זו רק בדו"ח שנה ראשונה במחקר שאושר לשנתיים, או בדו"ח שנה שניה במחקר שאושר לשלוש שנים