Hydrophobically-modified silica aerogels: Novel food-contact surfaces with bacterial anti-adhesion properties

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Abstract

In the context of food safety, contamination of food-contact surfaces with pathogenic bacteria is a global concern. This work investigates the potential of hydrophobically-modified, silica aerogel as a bacterial anti-adhesion food-contact surface. The bacterial anti-adhesion efficacies of hydrophobic silica aerogel, hydrophilic silica (negative control), and hydrophobic silica (positive control) were evaluated using dip inoculation with *Salmonella Typhimurium* LT2 and *Listeria innocua* NADC 2841 at 8.8 to 9.1 log CFU/mL. After rinsing, cells on these surfaces were enumerated by conventional plating as well as direct counting via scanning electron microscopy (SEM). Compared with the negative control, the positive control and silica aerogel led to a reduced number of salmonellae by 1.2 ± 0.1 log units (93.23 ± 0.91%) and by 3.1 ± 0.1 log units (99.93 ± 0.01%) respectively via plate counting (p < 0.05). The log reductions in the number of *L. innocua* were 1.3 ± 0.0 (94.82 ± 0.21%) and 3.0 ± 0.0 (99.91 ± 0.01%) for the positive control and silica aerogel, respectively via plate counting (p < 0.05). Additional bacterial proliferation studies revealed that bacterial anti-adhesion properties, not antibacterial effects, were responsible for the observed reductions. Overall, bacterial anti-adhesion property as well as other distinctive properties such as superior thermal insulation and ultra-lightweight make hydrophobically-modified silica aerogel an attractive candidate as a novel food-contact surface.

1. Introduction

Foodborne disease cases arising from the bacterial cross-contamination of food-contact surfaces and the subsequent cross-contamination of food products represent a significant concern for public health and have emerged as a global challenge (Akhtar, Sarker, & Hossain, 2014; Humphrey, 2004; Shi & Zhu, 2009). The sources of pathogenic bacteria contaminating food-contact surfaces are typically soil, water, contaminated food, equipment, animals, humans, and aerosols (Hall-Stoodley, Costerton, & Stoodley, 2004), *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter* spp., and *Shigella* spp. are bacterial pathogens that can exist on food and food-contact surfaces (Brooks & Flint, 2008; Chmielewski & Frank, 2003; Stepanovic, Cirkovic, Ranin, & Svacic-Vlahovic, 2004). Currently, there are a number of studies reporting the mechanisms for interaction of these microorganisms with common materials of food-contact surfaces such as stainless steel, glass, paper, high density polyethylene, polycarbonate, polyurethane, and polytetrafluoroethylene (PTFE, Teflon) (Abban, Jakobsen, & Jespersen, 2012; Beresford, Andrew, & Shama, 2001;

After attachment, pathogenic bacteria can survive on food-contact surfaces such as stainless steel for hours or days after initial contact (Wilks, Michels, 2003; Moore, Sheldon, & Jaykus, 2003). When good hygienic practices are applied, such as washing with hot water and soap, it is possible to reduce the number of viable pathogens on food-contact surfaces (Perez, Lucia, Cisneros-Zevallos, Castillo, & Taylor, 2012). However, if cleaning and sanitizing procedures are inadequate, multiple scenarios for bacterial contamination where food safety and quality is compromised emerge (Hoelzer et al., 2012). Furthermore, if food-contact surfaces are used and abraded with time, cleaning and sanitizing may be even more difficult due to the development of crevices and other rough surfaces on them, thereby resulting in bacterial attachment and potential cross-contamination of foods (Verran, Packer, Kelly, & Whitehead, 2010). In summary, there is a need to develop food-contact surfaces that robustly inhibit the attachment of pathogens.

Merian and Goldard (2012) have recently reviewed the emerging classes of nonfouling materials that have a potential for food applications. To this end, protein-repellent surfaces (Kingshott, Wei, Bagge-Ravn, Gadegaard, & Gram, 2003; Zhang et al., 2014), zwitterionic surfaces (Cheng, Zhang, Chen, Bryers, & Jiang, 2007), stimuli-responsive polymers (Cunliffe, Alarcon, Peters, Smith, & Alexander, 2003), biomimetic materials (e.g., lotus leaf, rice leaf, butterfly wing, fish scale, and shark skin) (Bixler & Bhushan, 2014), and amphiphilic surfaces (Krishnan et al., 2006) have been considered. In this study, we investigated the feasibility of hydrophobically-modified silica aerogel, an advanced material that can be prepared in an economical fashion, as a food-contact surface that could have anti-adherent activity against bacteria. Silica aerogel was selected based on reports on the use of functionalized silica mesoporous structures in several biomedical applications such as anti-fouling surfaces against proteins and cells. For instance, poly(carboxybetaine methacrylate) functionalized silica hydrogel was shown to resist protein (fibrinogen) adsorption (Beltran-Osuna et al., 2012). In another study, fluoroalkoxysilane coated structures involving silica colloids were found to reduce adhesion of Staphylococcus aureus and Pseudomonas aeruginosa (Privett et al., 2011). Hu et al. (2013) showed that composite structures involving poly(β-lactide) and silica nanoparticles exhibited anti-adhesion behaviour towards bacteria and cells. In addition, silica aerogels are very good thermal insulators (Gurav, Jung, Park, Kang, & Nadario, 2010; Schmidt & Schwertfeger, 1998). Such a property can be useful for some food-contact surfaces or process environments (e.g., post-lethality environments for the handling/packaging of fully cooked meats, produce cooling chambers, or other chilled food storage environments).

Gram-negative Salmonella Typhimurium LT2 and Salmonella Typhimurium 14082s and Gram-positive Listeria innocua NADC 2841 were utilized for studying the interactions of the developed food-contact surfaces with bacteria through dip inoculation. The bacterial attachment behaviour was evaluated using conventional plating and scanning electron microscopy (SEM). The surface and porosity properties of the silica aerogel were characterized using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, atomic force microscopy (AFM), contact angle measurements, Brunauer-Emmett-Teller (BET) analysis, and ellipsometry techniques.

2. Material and methods

2.1. Preparation of quartz and silica aerogel and their methylated versions

1 cm × 1 cm quartz (SiO2) slides (Ted Pella, Inc., Redding, CA, USA) were first rinsed with Milli-Q water (resistivity ≥ 18.2 MΩ cm) produced by an ultrapure water purification system (Milli-Q Advantage A10; EMD Millipore Corp., Billerica, MA, USA), and left dry at room temperature (20 °C). Subsequently, oxygen (O2; Brazos Valley Welding Supply, Inc., Bryan, TX) plasma treatment by CS-1701 reactive-ion etcher (RIE; Nordson March, Concord, CA, USA) was applied to remove organic adsorbates on surfaces and further clean the surfaces. In addition, plasma treatment is known to be an effective method for sanitizing surfaces from bacteria (Niemira, 2012; Zhang, Oh, Cisneros-Zevallos, & Akbulut, 2013), and can eliminate pre-existing bacteria, if any, present on surfaces.

After rinsing with sterile Milli-Q water again, these slides were used as the negative controls. In addition, some of the quartz slides were functionalized with trimethyloxil chloride (TMCS; Sigma—Aldrich Co., St. Louis, MO, USA) by placing the clean slides in 6% TMCS solution. The silanation reaction was allowed to take place for 24 h (Fig. 1). The slides were then rinsed with ethanol (200 proof; Koptec, King of Prussia, PA, USA) and purged with a stream of nitrogen (N2; Brazos Valley Welding Supply, Inc., Bryan, TX) for 10 min and left dry at room temperature (20 °C) before use (positive controls).

Silica (SiO2) aerogel was synthesized by the sol–gel polymerization of tetraethylorthosilicate (TEOS; Sigma—Aldrich Co., St. Louis, MO, USA) via hydrolysis and condensation reaction (Tamon, Kitamura, & Okazaki, 1998). Ammonium fluoride (NH4F; Sigma—Aldrich Co., St. Louis, MO, USA) was used as a hydrolysis catalyst, and ammonia hydroxide (NH4OH; Sigma—Aldrich Co., St. Louis, MO, USA) as a condensation catalyst. TEOS was dissolved in ethanol and the resultant solution was mixed with NH4F, NH4OH, and water to initiate the gelation. The reaction was allowed to take place for 24 h and the silica aerogel formed was dried using supercritical carbon dioxide (CO2; Brazos Valley Welding Supply, Inc., Bryan, TX) at the critical point (31.1 °C, 72.9 bar). This resulted in hydrophilic silica aerogel which was submerged in 6% TMCS solution for 24 h to functionalize silica surfaces with TMCS. Next, the functionalized silica aerogel was rinsed with hexane (Avantor Performance Materials, Inc., Center Valley, PA, USA) to eliminate excess TMCS and byproducts, and dried at 60 °C until hexane evaporated completely.

2.2. Characterization of quartz and aerogel

The chemical interactions of TMCS with silica materials (i.e., quartz and silica aerogel) were characterized by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. ATR-FTIR spectra were measured using an IRPrestige-21 (Shimadzu Corp., Kyoto, Japan) system and analysed using IRRsolution version 1.40 (Shimadzu Corp., Kyoto, Japan) software.

Surface topography of the samples was characterized using atomic force microscopy (AFM; Dimension Icon, Bruker, Santa Barbara, CA, USA). For AFM sample preparation, a stream of nitrogen gas was gently directed downward onto the surface of the samples to remove any potential dust, and then fixed on the stage with a super glue to decrease data noise due to its extremely lightweight. Topographical micrographs were obtained by using ScanAsyst™ mode in air. The silicon tip had the nominal spring constant 0.4 N/m, nominal tip radius 2 nm, and the nominal resonant frequency of 70 kHz.

In order to determine the surface hydrophobicity, static water contact angles were measured for different types of surfaces using...
ASAP2010 (Micromeritics Instrument Co., Norcross, GA, USA). The distribution of the hydrophobic silica aerogel. This was achieved by mine surface area, average pore diameter, and pore volume distribution via contact angle plug-in.

The Brasun-Emmett-Teller (BET) method was used to determine surface area, average pore diameter, and pore volume distribution of the hydrophobic silica aerogel. This was achieved by nitrogen adsorption isotherms at a temperature of 77 K by using ASAP2010 (Micromeritics Instrument Co., Norcross, GA, USA). The surface area was computed from N2 adsorption curves following the Barret-Joyner-Halenda (BJH) method (Barrett, Joyner, & Halenda, 1951).

The refractive index of silica aerogel was measured using an angle dependent ellipsometer (Nanofilm EP3-SE; Nanofilm Technology GmbH, Göttingen, Germany) under dry and wet conditions to calculate what fraction of nanopores of silica aerogel was filled with water upon water contact.

2.3. Chemical stability tests

Chemical stability of hydrophobic silica aerogel was monitored in deionized (DI) water (H2O) and in 10% hydrogen peroxide (H2O2, 30% solution; Avantor Performance Materials, Inc., Center Valley, PA, USA), a commonly used sanitizer in food industry, as a function of time. This was achieved through analysing aliquots collected from solutions containing submerged hydrophobic silica aerogel pieces using ATR-FTIR. These measurements were conducted at immersion times of 4 h, 3 days, 1 week, and 2 weeks for both DI water (H2O) and 10% hydrogen peroxide (H2O2) solutions.

2.4. Growth and preparation of microorganisms

Salmonella enterica subsp. enterica serovar Typhimurium str. LT2 (ATCC 700720; American Type Culture Collection, Manassas, VA, USA) and L. innocua NADC 2841 (NADC 2841; National Animal Disease Center, Ames, IA, USA) were obtained from the Center for Food Safety culture collection in the Department of Animal Science (Texas A&M University, College Station, TX, USA). Working cultures of S. Typhimurium LT2 were obtained by transferring a loopful of culture from a TSA slant containing 0.6% yeast extract (Becton, Dickinson and Co., Sparks, MD, USA) to 9.0 mL of TSB containing 0.6% yeast extract. The tubes for all strains were incubated aerobically without agitation at 37 °C for 24 h. After 24 h, a loopful of culture was transferred to fresh TSB (or TSB containing 0.6% yeast extract for L. innocua NADC 2841), and incubated aerobically for 24 h at 37 °C twice consecutively. The final concentration reached by S. Typhimurium LT2 and L. innocua NADC 2841 in the growth medium ranged from 8.8 to 9.1 log CFU/mL.

2.5. Inoculation of surfaces with bacterial organisms

For sterilization purposes, each sample i.e., hydrophilic nonporous silica (negative control), hydrophobic nonporous silica (positive control), and hydrophobic silica aerogel (nanoporous) was washed in 70% ethanol for 5 min and then rinsed in sterile Milli-Q water. After completion of the sterilization process, the absence of microorganisms was confirmed by SEM. Next, the samples were immersed in 9.0 mL bacterial suspensions (8.8–9.1 log CFU/mL) for 4 h at room temperature (20 °C). Then, the samples were gently removed from the bacterial suspension in a single vertical motion, and held vertically for 5 min to eliminate the remaining droplet so that drying effects were not superimposed on the adhesion effects. Finally, nitrogen gas was gently blown on the sample to further remove the thin liquid film. The treated samples were then isolated for counting attached bacterial cells. All of these experiments were carried out under sterile conditions in biological safety cabinet to prevent any contamination.

For the comparison purposes, the above mentioned dipping inoculation assay was also utilized for common food-contact materials such as polytetrafluoroethylene (PTFE, Teflon), polycarbonate, stainless steel, and glass. The disk shape samples (10 mm in diameter and 5 mm in height) were sterilized and inoculated as described above. All inoculation experiments were replicated four times.

2.6. Enumeration of surface-attached bacteria

As a direct counting approach, a scanning electron microscope (SEM; JSM-7500F JEOJ, Tokyo, Japan) was used to observe S. Typhimurium LT2 and L. innocua NADC 2841 on surfaces to quantify bacterial adhesion on various types of silica surfaces. In SEM experiments, a thin layer (15 nm) gold (Au) film was deposited on the bacteria adhered surfaces to ensure the scattering contrast and electrical conductivity required by SEM technique. The SEM micrographs were analysed by ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA) to quantify S. Typhimurium LT2.
and *L. innocua* NADC 2841 attachments. For statistical reliability, at least nine different areas of 100 μm × 100 μm from three different samples were observed to count number of attached bacteria.

We also enumerated bacteria on silica aerogel using pour plating approach. Briefly, silica aerogel samples (5 mm × 5 mm) inoculated by bacteria were vortex-mixed in sterile water for 10 min to detach bacteria from surfaces. Then, serial dilutions of the suspension containing detached bacteria were made and plated on TSA containing 0.1 g/mL of rifampicin (Sigma–Aldrich Co., St. Louis, MO, USA) for *S. Typhimurium* LT2 and TSA containing 6.0 μg/mL yeast extract for *L. innocua* NADC 2841. Bacterial densities were determined after 24 h of aerobic incubation at 37 °C. The plating experiments were replicated six times.

2.7. *Bacterial proliferation assay for investigating the relative importance of antibacterial activity and bacterial anti-adhesion*

After observing significant reductions in bacterial attachment on silica aerogel, we performed an additional assay to determine if bacterial anti-adhesion or antibacterial activity is responsible for the observed trends. Briefly, bacterial (*S. Typhimurium* LT2 and *L. innocua* NADC 2841) suspensions (8.8–9.1 log CFU/mL) were exposed to TMCS-functionalized silica aerogel through immersion or to 1% (v/v) bleach solution through mixing (positive control) for 4 h at room temperature (20 °C). Bacterial suspension without any exposure step was used as the negative control. Then, pour plating method was utilized by taking 1.0 mL of bacterial suspension from each solution to make serial dilutions and then by counting the total number of bacteria. These experiments were replicated three times for each condition.

2.8. *Characterization of thermal properties*

The thermal insulation behaviour was evaluated using a thermochromic film placed directly on top of the surface of interest (see [Supplementary Information, Section 1.1](#) for further details). The prepared thermochromic film was found to change its colour from blue to red at temperatures 40 °C or above. PTFE, polycarbonate, stainless steel, and glass samples having similar shape and dimensions to hydrophobic silica aerogel sample (10 mm in diameter and 5 mm in height) was manufactured. After the thermochromic film was placed on top of the manufactured samples, they were placed on a hot plate at a temperature of 100 °C for an hour. The time at which the colour of the thermochromic film on the samples changed from blue to red was used as a criterion of comparison for the thermal insulation behaviour.

2.9. *Statistical analysis*

Microbiological data from plate counts and SEM were transformed into logarithms of cells/mm². One-way and two-way analysis of variance (ANOVA) with Tukey’s post hoc test were used to determine significant differences between microbiological data from surface types and bacterial (*S. Typhimurium* LT2 and *L. innocua* NADC 2841) types (*p* < 0.05). All analyses were performed by using Microsoft Office Excel (Microsoft Corp., Redmond, WA, USA) statistical software packages.

3. Results and discussion

3.1. *Topography and porosity characteristics of silica aerogel*

Previous studies have shown that surface roughness can influence bacterial adhesion ([Canas et al., 2012; Truong et al., 2010](#)). Hence, we characterized the surface topography of the materials used in this study to better compare their adhesion behaviour. Fig. 2a–c displays AFM micrographs of hydrophilic nonporous quartz (*SiO₂*), hydrophobic nonporous quartz (*SiO₂*), and hydrophobic silica (*SiO₂*) aerogel (nanoporous) surfaces at a lower magnification. The analysis of the AFM micrographs revealed that the root-mean-square (RMS) roughness was 0.95 ± 0.05 nm, 1.44 ± 0.14 nm, and 104.01 ± 22.69 nm for hydrophilic quartz, hydrophobic quartz, and hydrophobic silica aerogel surfaces, respectively. This means that while hydrophobic silica aerogel surfaces were rougher than hydrophilic and hydrophobic quartz surfaces, the length scale of roughness for hydrophobic silica aerogel was still much smaller than diameter and length of bacteria used in this study (i.e., 1.0 μm–1.5 μm × 2 μm–6 μm) (Wang & Chen, 2009).

BET studies on hydrophobic silica aerogel revealed that the Barrett-Joyner-Halenda (BJH) average pore diameter of hydrophobic silica aerogel was 6.58 ± 0.59 nm, the BJH pore volume was 1.10 ± 0.10 cm³/g⁻¹, and the BET surface area was 761.54 ± 3.15 m²g⁻¹. These values are comparable with functionalized silica aerogel described in the literature (Hrubesh, 1998; Husing & Schubert, 1998; Pool, 1998). Herein, it is important to note that the length scale of the bacteria is much larger than the pore diameter of the silica aerogel (i.e., 1000 nm–1500 nm × 2000 nm–6000 nm versus ~7 nm), thereby inhibiting the penetration of bacteria into the hydrophobic silica aerogel.

3.2. *Characterization of functional groups on silica aerogel*

To confirm the methylation reaction on quartz and silica aerogel, ATR-FTIR spectroscopy was used. Fig. 3a and b displays ATR-FTIR spectra of pure (unreacted) TMCS, TMCS-functionalized (methylated) quartz, and TMCS-functionalized (methylated) silica aerogel surfaces. While the bare quartz and bare silica aerogel surfaces had no peak between 2800 cm⁻¹ and 3000 cm⁻¹, the hydrophobic (methylated) quartz had peaks at 2850 cm⁻¹, 2920 cm⁻¹, and 2967 cm⁻¹ and the hydrophobic (methylated) silica aerogel at 2900 cm⁻¹, 2962 cm⁻¹, and 2978 cm⁻¹. The presence of these peaks are attributed to symmetric and asymmetric C–H stretching from methyl groups formed upon the reaction of TMCS with silica surfaces. Unbound (free-standing) TMCS molecules had symmetric and asymmetric C–H stretching peaks at 2900 cm⁻¹ and 2962 cm⁻¹. The changes in C–H stretching behaviour are due to the substitution of Cl atoms by O atoms during methylation reaction and because of the transformation from the liquid state to crystalline state (Porter, Bright, Allara, & Chidsey, 1987). In addition, Si–Cl stretching vibration region ~620 cm⁻¹ only existed for TMCS (Du, Du, & George, 2007), supporting the methylation reaction shown in Fig. 1.

3.3. *Wetting characteristics of silica aerogel*

Hydrophilic materials tend to aggregate on hydrophilic surfaces (Chandler, 2005; Grant, Tiberg, & Ducker, 1998). While bacteria can adhere on both hydrophilic and hydrophobic surfaces, bacterial attachment tends to occur significantly more on hydrophilic surfaces ([Lima, Sao Jose, Andrade, Pires, & Ferreira, 2013](#)). Therefore, it is necessary to investigate the hydrophobicity of surfaces to better explain the bacterial attachment data. The static water contact angle measurements (Fig. 3c) revealed that while the neat quartz was hydrophilic (θ < 10.0°), methylated quartz (θ ≈ 95.5 ± 12°) and methylated silica aerogel (θ ≈ 132.4 ± 37°) were hydrophobic. The difference between the contact angles of methylated quartz and silica aerogel can be explained by previous studies showing that surfaces with different roughness, textures, or crystal structures often display a variation in water contact angle values although their surface chemistry is the same ([Anselme et al., 2010; Drellich & Miller, 1994; Wenzel, 1949](#)).
3.4. Chemical stability of silica aerogel

The potential toxicity, if any, of the developed silica aerogel surfaces is directly related to their ability to release chemicals from their surfaces through detachment, degradation, or decomposition. Hence, we investigated chemical integrity and stability of hydrophobic silica aerogel in DI water (H₂O) and 10% hydrogen peroxide (H₂O₂) as a function of time using ATR-FTIR spectroscopy. As shown in Fig. 4, the spectroscopic analysis revealed that solutions containing submerged silica aerogel had no free chemicals within the detection limit of 1 ppm at least for two weeks.

3.5. Bacterial attachment behaviour of silica aerogel

Fig. 5a–c shows the SEM micrographs of three different types of silica materials described above following inoculation and attachment of S. Typhimurium LT2. The pristine quartz surface (hydrophilic, negative control) supported the greatest bacterial adhesion with a mean density of 5.6 ± 0.0 log cells/mm² (Fig. 5d). When the quartz was methylated (hydrophobized) i.e., positive control, the bacterial adhesion decreased to a mean density of 4.6 ± 0.1 log cells/mm² which corresponds to 90.38 ± 2.75% of reduction. Bacterial adhesion on hydrophobic (methylated) silica aerogel surfaces led to a mean density of 2.6 ± 0.3 log cells/mm² which achieved a relatively high reduction of 99.91 ± 0.05%. One-way ANOVA analysis showed that the difference in the adhesion of S. Typhimurium LT2 with respect to the sample type is statistically significant (p < 0.05). In addition to direct counting via scanning electron microscopy (SEM), pour plating was used to enumerate microorganisms on these samples (Fig. 5e). Plating studies showed that compared with the negative control (pristine quartz), the positive control (methylated quartz) and silica aerogel led to a
reduced number of salmonellae by 1.2 ± 0.1 log units (93.23 ± 0.91%) and by 3.1 ± 0.1 log units (99.93 ± 0.01%), respectively. According to one-way ANOVA test, these values were significantly different at \( p < 0.05 \) level. We note that the log reduction values were smaller in plating studies, presumably due to the lack of bacterial growth step in direct counting studies via SEM. Similar reduction trends were also observed for pathogenic \( S. \) enterica subsp. enterica serovar Typhimurium str. 14028s (see Supplementary Information, Section 1.2 and Figure S1 for further details).

To determine if the above observed trends also take place for Gram-positive bacteria, we repeated direct counting via SEM and plating experiments using \( L. \) innocua NADC 2841. Fig. 6a–c shows the SEM micrographs of \( L. \) innocua NADC 2841 attachment on three different types of silica materials described above. While hydrophilic quartz surfaces yielded a mean bacterial density of 5.8 ± 0.1 log cells/mm², hydrophobic quartz surfaces had a reduced number of bacteria attached with a mean density of 4.7 ± 0.0 log cells/mm², corresponding to 92.01 ± 1.03% reduction (Fig. 6d). Hydrophobic silica aerogel surfaces displayed much lower degree of bacterial attachment with a mean density of 2.5 ± 0.2 log cells/mm², indicating 99.94 ± 0.03% reduction in comparison to the hydrophilic quartz (negative control). In pour plating studies, using the negative control (the pristine quartz) as reference, the log reduction values were calculated to be 1.3 ± 0.0 log units (94.82 ± 0.21%) and 3.0 ± 0.0 log units (99.93 ± 0.01%).
log units (99.91 ± 0.01%) for methylated quartz (positive control) and silica aerogel, respectively (Fig. 6e). One-way ANOVA analysis indicated that the difference in the number of *L. innocua* NADC 2841 with respect to sample type was statistically significant (*p* < 0.05) for both direct counting and traditional plating approaches. The comparison of microbiological data on three types of silica surfaces with respect to the bacterial types via two-way ANOVA indicated that for all surface types, the attachment behaviour of Gram-negative (*S. Typhimurium* LT2) and Gram-positive (*Listeria NADC* 2841) on these was not significantly different (*p* > 0.05).

Bacteria with hydrophobic cell surface tend to adhere more extensively on hydrophobic material surfaces while those with hydrophilic properties prefer hydrophilic surfaces (Hogt, Dankert, Devries, & Feijen, 1983; Satou, Satou, Shintani, & Okuda, 1988). Given the contact angles of water on *S. Typhimurium* and *L. innocua* NADC 2841 are between 26° and 36°, thus fairly hydrophilic, the reduction in the bacterial adhesion on the hydrophobic quartz surfaces in comparison to the hydrophilic ones is consistent with the above-mentioned phenomena (Dickson & Koolmarae, 1989; Woodling & Moraru, 2005). However, the complete elimination of bacterial attachment upon changing the surface from hydrophobic quartz to hydrophobic silica aerogel cannot be explained solely by the hydrophobic effect, especially given both surfaces were functionalized with the same chemical group.

Because tail groups of TMCS are methyl groups, acid-base, hydrogen-bond, and specific ligand-receptor types of interactions between TMCS and bacteria are non-existing. Therefore, van der Waals interactions are expected to primarily govern the thermodynamics of bacterial adhesion on methylated quartz and methylated silica aerogel surfaces. The strength of van der Waals forces is directly related to the refractive index of interacting materials and dispersing medium (Israelachvili, 2011). For a given bacteria and aqueous medium, a lower refractive index of substrate will lead to a decrease in attractive van der Waals interactions between the substrate and bacteria (see Supplementary Information, Section 1.3 for further details). Due to their nanoporous nature, silica aerogels can have much lower refractive index than nonporous silica materials (i.e., quartz) (Yang, Choi, Hyun, & Park, 1999). Using spectroscopic ellipsometry, we found that for the methylated silica aerogel prepared, the refractive index was 1.008 ± 0.001, which is indeed much smaller than the refractive index of nonporous silica materials, 1.45—1.55. Hence, the superior ability of hydrophobic silica aerogel to inhibit bacterial attachment is attributed to the reduction of attractive van der Waals interactions due to their nanoporous nature.

### 3.6. Comparison of bacterial attachment of silica aerogel and common food-contact materials

For a direct comparison purpose, we carried out identical dipping inoculation tests with common food-contact materials such as PTFE, polycarbonate, stainless steel, and glass as well as silica aerogel. Fig. 7 displays SEM micrographs of hydrophobic (methylated) silica aerogel and common food-contact materials after inoculation by *S. Typhimurium* LT2 and *L. innocua* NADC 2841 bacterial suspensions. Methylated silica aerogel clearly displayed superior anti-adhesion performance in comparison to PTFE, polycarbonate, stainless steel, and glass. Considering that PTFE is very hydrophobic surface, improvements in inhibition of bacterial attachment behaviour for silica aerogel also support the above-mentioned discussion that hydrophobic effect cannot be solely responsible for the observed adhesion trends. Overall, these promising findings indicate a high inhibition efficiency of hydrophobic silica aerogel.

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**Fig. 6.** SEM micrographs of (a) hydrophilic quartz, (b) hydrophobic quartz, and (c) hydrophobic silica aerogel (black circles indicate attached bacteria) after inoculation with *L. innocua* NADC 2841. Panel (d) relates the number of bacteria per unit area (mm²) remaining on surfaces (a logarithmic scale is chosen for the y-axis). Bacterial adhesion was statistically different between all surfaces as determined by mean numbers of attached cells following counting (*p* < 0.05). (e) Microbiological data obtained by pour plating method. Different letters indicate statistically significant difference (*p* < 0.05).
against bacterial adhesion and also show how effectively hydrophobic silica aerogel can prevent bacteria attachment compared to common food-contact materials.

3.7. Screening of hydrophobic silica aerogel for absence of antibacterial activity

To determine if bacterial anti-adhesion or antibacterial property is responsible for the log reduction trends in the bacterial attachment on silica aerogel, we carried out bacterial growth studies in the presence of silica aerogel (Table 1). In comparison to bacterial suspension without any treatment, for bacterial suspension with 1% bleach solution, a log reduction of $8_{10}^9$ was observed. On the other hand, there was no change in the number of bacteria growing in the presence of silica aerogel (see Supplementary Information, Figure S2 for further details). Overall these findings indicate that silica aerogel displays no antibacterial activity, and hence, bacterial anti-adhesion is indeed responsible for the observed inhibition trends in bacterial adhesion.

3.8. Measurement of thermal insulation properties

The thermal insulation behaviour of methylated silica aerogel and commonly used food-contact materials were compared using a thermochromic film that changes its colour from blue (in web version) to red (in web version) at temperatures 40 °C or above (Fig. 8). It was found that after keeping all of the surfaces on hot plate for 1 h at 100 °C, thermochromic film on all materials except hydrophobic silica aerogel changed its colour blue to red. These findings indicate that methylated silica aerogel displayed superior thermal insulating performance in comparison to common food-contact surfaces. In addition, by measuring the temperatures of the silica aerogel surfaces and hot plate and the time of heating, we estimated the thermal conductivity of hydrophobic silica aerogel to be 0.052 ± 0.015 W/m·K, which is about an order of magnitude smaller than typical plastics and ceramics (Callister & Rethwisch, 2012; Rao, Pajonk, & Haranath, 2001; Singh, Burgess, & Singh, 2008).

### Table 1

Comparison of bacterial proliferation behaviour in the absence and in the presence of TMCS-silica aerogel sample, and in the presence of 1% bleach solution against S. Typhimurium LT2 and L. innocua NADC 2841. Significant reduction was only observed for 1% bleach solution.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Bacterial suspension alone (control)</th>
<th>Bacterial suspension with TMCS-Silica aerogel</th>
<th>Bacterial suspension with 1% bleach solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhimurium LT2</td>
<td>$3.7 \times 10^8$ CFU/mL$^a$</td>
<td>$3.2 \times 10^8$ CFU/mL$^a$</td>
<td>&lt;1 (zero)</td>
</tr>
<tr>
<td>L. innocua NADC 2841</td>
<td>$1.1 \times 10^9$ CFU/mL$^a$</td>
<td>$1.2 \times 10^9$ CFU/mL$^a$</td>
<td>&lt;1 (zero)</td>
</tr>
</tbody>
</table>

$^a$ Values of bacterial population (CFU/mL) after 4 h of exposure.

Fig. 7. Comparison of bacterial (S. Typhimurium LT2 and L. innocua NADC 2841) adhesion behaviour on (a), (b) hydrophobic (methylated) silica aerogel (black circles indicate attached bacteria) and (c–j) common food-contact materials: PTFE (hydrophobic), polycarbonate, stainless steel, and glass.

4. Conclusions

In this work, we showed that the attachments of S. Typhimurium LT2, L. innocua NADC 2841, and S. Typhimurium 14028s on presence of silica aerogel (see Supplementary Information, Figure S2 for further details). Overall these findings indicate that silica aerogel displays no antibacterial activity, and hence, bacterial anti-adhesion is indeed responsible for the observed inhibition trends in bacterial adhesion.
methylated (hydrophobic) silica aerogel were significantly inhibited due to its bacterial anti-adhesion properties. Direct comparative studies indicated that methylated silica aerogel can prevent bacterial attachment much more effectively compared to common food-contact materials such as polycarbonate, stainless steel, glass as well as hydrophobic PTFE. The superior ability of methylated silica aerogel to inhibit bacterial attachment is attributed to its nanoporosity and porosity-induced reduction in the attractive van der Waals interactions between silica aerogel and bacteria. Overall, combining bacterial anti-adhesion properties of hydrophobic silica aerogel with their other unique properties such as thermal insulation and ultra-lightweight can open up new avenues in the design of food-contact surfaces.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2014.12.029.

References


![Fig. 8. Comparison of thermal insulation properties of (a) hydrophobic silica aerogel (TMCS-Silica aerogel) and (b–e) common food-contact materials: PTFE (hydrophobic, Teflon) polycarbonate, stainless steel, and glass.](image)
