Microorganism adhesion using silicon dioxide: An experimental study

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ABSTRACT

In this study, spectrophotometry was used to measure changes in the absorbance properties of yeast, Gram-positive, and Gram-negative bacteria after their attachment to silicon dioxide microparticles (silica). The goal of this study was to determine whether spectrophotometry is an effective method to distinguish these microorganisms from one another and determine whether they have an affinity for silicon dioxide. The experiments were performed by examining the light absorption properties of yeast, Gram-positive and Gram-negative bacteria in a spectrophotometer, both with and without silicon dioxide microparticles. The experiments produced a number of promising results. First, the spectrophotometer graphs of yeast were noticeably different from those of both Gram-positive and Gram-negative bacteria. Second, the absorption of light in both Gram-positive and Gram-negative bacteria occurred at near infrared range (700–1500 nm) and, unlike yeast, the wavelengths increased when silicon dioxide microparticles were added to the suspension. When silicon dioxide microparticles were added to yeast, the absorption of light decreased during the entire wavelength interval of the spectrophotometer measurement. These results indicate that bacteria have an affinity for silicon dioxide, and that spectrophotometry may be used to distinguish yeast from bacteria and, possibly, different bacterial types from one another.

1. Introduction

A key factor in microorganism pathogenesis is its adhesion to surfaces. Binding to surfaces gives a microorganism an opportunity to grow and form biofilms, which are collections of microorganisms held together with a self-produced extracellular matrix [1, 2]. Hence, examining how adhesion occurs and determining the factors that affect adhesive ability may help in better understanding microorganism pathogenesis. Studies have suggested that silicon dioxide can be used to investigate the attracting forces between microorganisms and inorganic surfaces [3]. Therefore, in this study, spectrophotometry was used to examine the adhesion of microorganisms to inorganic surfaces. A spectrophotometer is a common device that has been used for research and diagnostic purposes since 1940 and measures the transmission and absorption of light at different wavelengths [4]. It is used in medicine to determine the concentration of substances in body fluids or following the addition of an enzyme-linked colouring agent [5, 6].

In one study in which densitometry was used to analyse the interactions between microorganisms and silica, it was found that decreases in turbidity occurred more rapidly when silicon dioxide was added to both bacteria and yeast in comparison to without silicon dioxide [7]. Another study demonstrated that silica is a more favourable surface for the development of biofilms than cellulose films, which are biotic surfaces [8].

An examination of microorganism adhesion is important given that adhesion plays a key role in pathogenesis. Furthermore, determining how microorganisms specifically interact with silicon dioxide is clinically important, as it is commonly used in the production of small joint prostheses, dental cements, and as a coating for other types of implantable prostheses [9, 10, 11]. While infection of a joint prosthesis is uncommon, it is the most severe complication that can occur after prosthetic implantation [12]. Additionally, finding new and more rapid methods for determining the type of microorganism causing an infection is important for the clinical management of infectious diseases.

Several mechanisms determine the adhesion properties of microorganisms. Adhesion pili play a vital role in allowing bacteria to stick to body surfaces. They are mostly seen on Gram-negative bacteria, although a few Gram-positive bacteria have them as well [13]. Van der Waals forces are another mechanism that can affect the surface-adhesion abilities of microorganisms. These are intermolecular forces that attract...
adjacent molecules to one another [14]. The Zeta potential can also affect microorganism adhesion. This is the potential of the Stern plane of an electrical double layer that covers a cell in a solution. It is typically a negative charge for bacteria and provides a repulsive force from a negatively charged cell or particle [15]. Studies have indicated that silicon dioxide can increase the negative charge of a bacterial membrane when it is adsorbed onto a cell surface [16].

A number of studies have suggested that bacteria have an affinity for silicon dioxide [2, 16, 17]. Therefore, one aim of this study was to examine whether microorganisms have an affinity for silicon dioxide and, if so, what types of microorganisms exhibit this affinity. A further aim was to investigate the possibility of distinguishing one microorganism from another using spectrophotometry. Such a possibility has been studied previously using Escherichia coli and Bacillus globigii—with promising results [18]. This study included 4 yeasts, and 5 Gram-positive and 8 Gram-negative bacteria.

2. Materials and methods

2.1. Preparation of bacterial, yeast, and silicon dioxide suspensions

This study was conducted in the Microbiology laboratory of the Traumatology and Orthopaedics Hospital in Latvia. To prepare a 4 McFarland unit silicon dioxide suspension, Sigma-Aldrich silicon dioxide microparticles with a mean particle size of 9–13 μm and density of 1.05–1.15 g/ml were added to distilled water. Samples were taken from the suspension, placed in a test tube, and its turbidity was measured using a DEN-1 densitometer. If needed, additional silicon dioxide was added to the distilled water until the densitometer showed a turbidity of 4 ± 0.1 McFarland units. Samples were then taken from reference cultures of the microorganisms and placed in test tubes filled with 8 ml of distilled water. In total, 17 microorganisms were examined: 5 reference cultures of Gram-positive bacteria (Enterococcus faecalis ATCC (American Type Culture Collection) 29212, Staphylococcus epidermidis ATCC 12228, MSSA (Methicillin-sensitive Staphylococcus aureus) ATCC 25923, MRSA (Methicillin-resistant Staphylococcus aureus) ATCC 33591, and Bacillus spizizenii ATCC 66338); 8 reference cultures of Gram-negative bacteria (Proteus mirabilis ATCC 43071, Enterobacter aerogenes ATCC 13048, Salmonella enteritidis ATCC 13076, Pseudomonas aeruginosa ATCC 27853, Citrobacter freundii ATCC 438764, Klebsiella pneumoniae ATCC 700603, Moraxella catarrhalis ATCC 25238, and Escherichia coli ATCC 25922); and 4 pure cultures of yeasts: Kluyveromyces marxianus, Candida glabrata, Candida krusei, Candida albicans ATCC 10231). The OD600 (optical density at 600 nm) of each microorganism at 4 McFarland units is shown in Table 1. The suspensions were placed inside the densitometer to measure their turbidity. If needed, additional microorganism samples from the reference cultures were added until the densitometer showed a turbidity of 4 ± 0.1 McFarland units.

2.2. Microorganism samples with and without silicon dioxide suspensions

Seventeen cuvettes were filled with 4 ml of the 4 ± 0.1 McFarland unit suspensions of each microorganism. Another 17 cuvettes were filled with 3.5 ml of the 4 ± 0.1 McFarland unit microorganism suspensions. We added 0.5 ml of the 4 ± 0.1 McFarland unit silicon dioxide suspension to these cuvettes. One cuvette was filled with 4 ml of distilled water and another was filled with a 4 ml of 4 ± 0.1 McFarland unit silicon dioxide suspension.

2.3. Calibration of the spectrophotometer and measuring the absorbance of microorganisms

Samples were measured using a Shimadzu-1600 spectrophotometer (Kyoto, Japan: Shimadzu Corp.). The spectrophotometer’s mode was set to ‘spectrum’. A cuvette with distilled water was placed in the spectrophotometer and the ‘base core’ option was selected. This ensured that the light passing through the distilled water at all measured wavelengths was 100% of the light detected by the photodetector, i.e., 0 absorbance units. The spectrophotometer was set to measure absorbance at a wavelength interval of 285–1100 nm. The speed of the measurements was set to ‘fast’ and the distance between each measured wavelength was set to 1 nm. The initial wavelength of the measurements was 1100 nm and then lowered to 285 nm. To prevent settling, cuvettes were gently tilted several times before being placed in the spectrophotometer. Each sample at a 285–1100 nm wavelength was measured once. Measurements were repeated using a wavelength interval of 285–700 nm. The difference between each measured wavelength was set to 1 nm. Each sample was measured once, and each measurement for the wavelength interval of 285–1100 nm took 57 s. For the 285–700 nm wavelength interval, the measurement was completed in 30 s.

2.4. Comparison of microorganisms

All results were converted from an SPc (spectrum) format to a CSV (comma-separated values) format in the spectrophotometer. Each microorganism measurement contained the wavelengths and the absorbance units at the current wavelength (Supplementary content: Results with a wavelength of 285–700 nm, Results with a wavelength of 285–1100 nm, Description of classification in Excel files). All absorbance measurements of the specific microorganisms within the wavelength interval of 285–1100 nm, both with and without silicon dioxide microparticles, were compared to all other study microorganisms using a two-sample t-test assuming unequal variances. Alpha was set to 0.05 and a hypothesized mean difference set to 0. The comparisons, tables and graphs were done in Microsoft Excel (2016). Comparisons were repeated for measurements at 285–1100 nm with silicon dioxide microparticles, at 285–700 nm without silicon dioxide microparticles, and at 285–700 nm with silicon dioxide microparticles. A significant difference was assumed if p < 0.05.

3. Results

3.1. Microorganism comparisons at 285–1100 nm

Table 2 presents the results of comparisons between the microorganisms at 285–1100 nm without silicon dioxide. Assuming significance at p < 0.05, it was possible to distinguish one microorganism from another in 99/136 (72.8%) of all the comparisons. A significant difference was found in 52/52 (100%) of the yeast to bacteria comparisons,
Table 2 (continued)

| Compared Microorganisms                                      | p value          |
|--------------------------------------------------------------|------------------|
| Staphylococcus epidermidis compared to Enterococcus faecalis | 0.914399         |
| Staphylococcus epidermidis compared to MSSA                  | <0.000001        |
| Staphylococcus epidermidis compared to MRSA                  | 0.000007         |
| Staphylococcus epidermidis compared to Bacillus subtilis     | <0.000001        |
| Staphylococcus epidermidis compared to Proteus mirabilis     | 0.660184         |
| Staphylococcus epidermidis compared to Enterobacter aerogenes| 0.779679         |
| Staphylococcus epidermidis compared to Salmonella enteritidis| 0.854942         |
| Staphylococcus epidermidis compared to Pseudomonas aeruginosa| 0.046329         |
| Staphylococcus epidermidis compared to Citrobacter freundii  | 0.543804         |
| Staphylococcus epidermidis compared to Klebsiella pneumoniae | <0.000001        |
| Staphylococcus epidermidis compared to Morganella catarrhalis| 0.045225         |
| Staphylococcus epidermidis compared to Escherichia coli      | 0.071304         |
| Enterococcus faecalis compared to MSSA                       | <0.000001        |
| Enterococcus faecalis compared to MRSA                       | 0.000021         |
| Enterococcus faecalis compared to Bacillus subtilis          | <0.000001        |
| Enterococcus faecalis compared to Proteus mirabilis          | 0.611955         |
| Enterococcus faecalis compared to Enterobacter aerogenes     | 0.711759         |
| Enterococcus faecalis compared to Salmonella enteritidis    | 0.788713         |
| Enterococcus faecalis compared to Pseudomonas aeruginosa     | 0.050461         |
| Enterococcus faecalis compared to Citrobacter freundii       | 0.507301         |
| Enterococcus faecalis compared to Klebsiella pneumoniae      | <0.000001        |
| Enterococcus faecalis compared to Morganella catarrhalis     | 0.051130         |
| Enterococcus faecalis compared to Escherichia coli           | 0.076582         |
| MSSA compared to MRSA                                        | 0.182256         |
| MSSA compared to Bacillus subtilis                           | 0.001273         |
| MSSA compared to Proteus mirabilis                          | <0.000001        |
| MSSA compared to Enterobacter aerogenes                     | <0.000001        |
| MSSA compared to Salmonella enteritidis                     | <0.000001        |
| MSSA compared to Pseudomonas aeruginosa                      | 0.000462         |
| MSSA compared to Citrobacter freundii                       | <0.000001        |
| MSSA compared to Klebsiella pneumoniae                       | <0.000001        |
| MSSA compared to Morganella catarrhalis                      | 0.000077         |
| MSSA compared to Escherichia coli                            | 0.000063         |
| MRSA compared to Bacillus subtilis                           | 0.000005         |
| MRSA compared to Proteus mirabilis                          | 0.000213         |
| MRSA compared to Enterobacter aerogenes                      | 0.000125         |
| MRSA compared to Salmonella enteritidis                      | 0.000009         |
| MRSA compared to Pseudomonas aeruginosa                      | 0.031052         |
| MRSA compared to Citrobacter freundii                       | 0.000032         |
| MRSA compared to Klebsiella pneumoniae                       | <0.000001        |
| MRSA compared to Morganella catarrhalis                      | 0.013127         |
| MRSA compared to Escherichia coli                            | 0.010382         |
| Bacillus subtilis compared to Proteus mirabilis              | <0.000001        |
| Bacillus subtilis compared to Enterobacter aerogenes         | <0.000001        |
| Bacillus subtilis compared to Salmonella enteritidis         | <0.000001        |
| Bacillus subtilis compared to Pseudomonas aeruginosa         | <0.000001        |
| Bacillus subtilis compared to Citrobacter freundii           | <0.000001        |
| Bacillus subtilis compared to Klebsiella pneumoniae          | 0.017308         |
| Bacillus subtilis compared to Morganella catarrhalis         | <0.000001        |
| Bacillus subtilis compared to Escherichia coli               | <0.000001        |
| Proteus mirabilis compared to Enterobacter aerogenes         | 0.891944         |
| Proteus mirabilis compared to Salmonella enteritidis         | 0.815833         |
| Proteus mirabilis compared to Pseudomonas aeruginosa         | 0.147890         |
| Proteus mirabilis compared to Citrobacter freundii           | 0.888902         |
| Proteus mirabilis compared to Klebsiella pneumoniae          | <0.000001        |
| Proteus mirabilis compared to Morganella catarrhalis         | 0.161325         |
| Proteus mirabilis compared to Escherichia coli               | 0.921673         |
| Enterobacter aerogenes compared to Salmonella enteritidis    | 0.922302         |
| Enterobacter aerogenes compared to Pseudomonas aeruginosa    | 0.114523         |
Table 2 (continued)

| Microorganisms Compared | p value |
|-------------------------|---------|
| Enterobacter aerogenes compared to Citrobacter freundii | 0.775902 |
| Enterobacter aerogenes compared to Klebsiella pneumoniae | <0.000001 |
| Enterobacter aerogenes compared to Moraxella catarrhalis | 0.123126 |
| Enterobacter aerogenes compared to Escherichia coli | 0.169519 |
| Salmonella enteritidis compared to Pseudomonas aeruginosa | 0.096149 |
| Salmonella enteritidis compared to Citrobacter freundii | 0.702467 |
| Salmonella enteritidis compared to Klebsiella pneumoniae | <0.000001 |
| Salmonella enteritidis compared to Moraxella catarrhalis | 0.102396 |
| Salmonella enteritidis compared to Escherichia coli | 0.142982 |
| Pseudomonas aeruginosa compared to Citrobacter freundii | 0.186131 |
| Pseudomonas aeruginosa compared to Klebsiella pneumoniae | <0.000001 |
| Pseudomonas aeruginosa compared to Moraxella catarrhalis | 0.878623 |
| Pseudomonas aeruginosa compared to Escherichia coli | 0.778446 |
| Citrobacter freundii compared to Klebsiella pneumoniae | <0.000001 |
| Citrobacter freundii compared to Moraxella catarrhalis | 0.205258 |
| Citrobacter freundii compared to Escherichia coli | 0.270597 |
| Klebsiella pneumoniae compared to Moraxella catarrhalis | <0.000001 |
| Klebsiella pneumoniae compared to Escherichia coli | <0.000001 |
| Moraxella catarrhalis compared to Escherichia coli | 0.886808 |

Table 3 (continued)

| Microorganisms Compared | p value |
|-------------------------|---------|
| Candida glabrata compared to MSSA | <0.000001 |
| Candida glabrata compared to Bacillus subtilis | <0.000001 |
| Candida glabrata compared to Proteus mirabilis | <0.000001 |
| Candida glabrata compared to Enterobacter aerogenes | <0.000001 |
| Candida glabrata compared to Salmonella enteritidis | <0.000001 |
| Candida glabrata compared to Pseudomonas aeruginosa | <0.000001 |
| Candida glabrata compared to Citrobacter freundii | <0.000001 |
| Candida glabrata compared to Klebsiella pneumoniae | <0.000001 |
| Candida glabrata compared to Moraxella catarrhalis | <0.000001 |
| Candida glabrata compared to Escherichia coli | <0.000001 |
| Candida glabrata compared to MSSA | <0.000001 |
| Candida krusei compared to MSSA | <0.000001 |
| Candida krusei compared to Bacillus subtilis | <0.000001 |
| Candida krusei compared to Proteus mirabilis | <0.000001 |
| Candida krusei compared to Enterobacter aerogenes | <0.000001 |
| Candida krusei compared to Salmonella enteritidis | <0.000001 |
| Candida krusei compared to Pseudomonas aeruginosa | <0.000001 |
| Candida krusei compared to Citrobacter freundii | <0.000001 |
| Candida krusei compared to Klebsiella pneumoniae | <0.000001 |
| Candida krusei compared to Moraxella catarrhalis | <0.000001 |
| Candida krusei compared to Escherichia coli | <0.000001 |
| Staphylococcus epidermidis compared to Enterococcus faecalis | 0.037622 |
| Staphylococcus epidermidis compared to MSSA | 0.000184 |
| Staphylococcus epidermidis compared to MRSA | 0.722643 |
| Staphylococcus epidermidis compared to Bacillus subtilis | <0.000001 |
| Staphylococcus epidermidis compared to Proteus mirabilis | 0.007244 |
| Staphylococcus epidermidis compared to Enterobacter aerogenes | 0.000257 |
| Staphylococcus epidermidis compared to Salmonella enteritidis | 0.000004 |
| Staphylococcus epidermidis compared to Pseudomonas aeruginosa | 0.140400 |
| Staphylococcus epidermidis compared to Citrobacter freundii | 0.000026 |
| Staphylococcus epidermidis compared to Klebsiella pneumoniae | 0.00552 |
| Staphylococcus epidermidis compared to Moraxella catarrhalis | 0.071354 |
| Staphylococcus epidermidis compared to Escherichia coli | 0.654009 |
| Enterococcus faecalis compared to MSSA | <0.000001 |
| Enterococcus faecalis compared to MRSA | 0.027201 |
| Enterococcus faecalis compared to Bacillus subtilis | <0.000001 |
| Enterococcus faecalis compared to Proteus mirabilis | 0.535533 |
| Enterococcus faecalis compared to Enterobacter aerogenes | 0.116586 |
| Enterococcus faecalis compared to Salmonella enteritidis | 0.012177 |
| Enterococcus faecalis compared to Pseudomonas aeruginosa | 0.649447 |
| Enterococcus faecalis compared to Citrobacter freundii | 0.043448 |
| Enterococcus faecalis compared to Klebsiella pneumoniae | 0.000002 |
| Enterococcus faecalis compared to Moraxella catarrhalis | 0.772104 |
| Enterococcus faecalis compared to Escherichia coli | 0.131859 |
| MSSA compared to MRSA | 0.902679 |
| MSSA compared to Bacillus subtilis | 0.001238 |
| MSSA compared to Proteus mirabilis | <0.000001 |
| MSSA compared to Enterobacter aerogenes | <0.000001 |
| MSSA compared to Salmonella enteritidis | <0.000001 |
| MSSA compared to Pseudomonas aeruginosa | <0.000002 |
| MSSA compared to Citrobacter freundii | <0.000001 |
| MSSA compared to Klebsiella pneumoniae | 0.237971 |
| MSSA compared to Moraxella catarrhalis | <0.000001 |
| MSSA compared to Escherichia coli | 0.001013 |
| MRSA compared to Bacillus subtilis | <0.000001 |
| MRSA compared to Proteus mirabilis | 0.005793 |
| MRSA compared to Enterobacter aerogenes | 0.002271 |
| MRSA compared to Salmonella enteritidis | 0.000006 |

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Table 3 (continued)

| Compared Microorganisms          | p value  |
|----------------------------------|----------|
| MRSA compared to Pseudomonas aeruginosa | 0.096462 |
| MRSA compared to Citrobacter freundii | 0.000036 |
| MRSA compared to Klebsiella pneumoniae | 0.036991 |
| MRSA compared to Moraxella catarrhalis | 0.050037 |
| MRSA compared to Escherichia coli | 0.461519 |
| Bacillus spizizenii compared to Proteus mirabilis | <0.000001 |
| Bacillus spizizenii compared to Enterobacter aerogenes | <0.000001 |
| Bacillus spizizenii compared to Salmonella enteritidis | <0.000001 |
| Bacillus spizizenii compared to Pseudomonas aeruginosa | <0.000001 |
| Bacillus spizizenii compared to Citrobacter freundii | <0.000001 |
| Bacillus spizizenii compared to Klebsiella pneumoniae | 0.000001 |
| Bacillus spizizenii compared to Moraxella catarrhalis | <0.000001 |
| Bacillus spizizenii compared to Escherichia coli | <0.000001 |
| Proteus mirabilis compared to Enterobacter aerogenes | 0.347264 |
| Proteus mirabilis compared to Salmonella enteritidis | 0.061169 |
| Proteus mirabilis compared to Pseudomonas aeruginosa | 0.301413 |
| Proteus mirabilis compared to Citrobacter freundii | 0.173284 |
| Proteus mirabilis compared to Klebsiella pneumoniae | <0.000001 |
| Proteus mirabilis compared to Moraxella catarrhalis | 0.362275 |
| Proteus mirabilis compared to Escherichia coli | 0.036913 |
| Enterobacter aerogenes compared to Salmonella enteritidis | 0.348114 |
| Enterobacter aerogenes compared to Pseudomonas aeruginosa | 0.053585 |
| Enterobacter aerogenes compared to Citrobacter freundii | 0.666695 |
| Enterobacter aerogenes compared to Klebsiella pneumoniae | <0.000001 |
| Enterobacter aerogenes compared to Moraxella catarrhalis | 0.061709 |
| Enterobacter aerogenes compared to Escherichia coli | 0.002674 |
| Salmonella enteritidis compared to Pseudomonas aeruginosa | 0.004847 |
| Salmonella enteritidis compared to Citrobacter freundii | 0.561159 |
| Salmonella enteritidis compared to Klebsiella pneumoniae | <0.000001 |
| Salmonella enteritidis compared to Moraxella catarrhalis | 0.004938 |
| Salmonella enteritidis compared to Escherichia coli | 0.000095 |
| Pseudomonas aeruginosa compared to Citrobacter freundii | <0.000001 |
| Pseudomonas aeruginosa compared to Klebsiella pneumoniae | <0.000001 |
| Pseudomonas aeruginosa compared to Moraxella catarrhalis | 0.852092 |
| Pseudomonas aeruginosa compared to Escherichia coli | 0.327486 |
| Citrobacter freundii compared to Klebsiella pneumoniae | <0.000001 |
| Citrobacter freundii compared to Moraxella catarrhalis | 0.019583 |
| Citrobacter freundii compared to Escherichia coli | 0.000489 |
| Klebsiella pneumoniae compared to Moraxella catarrhalis | 0.000008 |
| Klebsiella pneumoniae compared to Escherichia coli | 0.002762 |
| Moraxella catarrhalis compared to Escherichia coli | <0.000001 |

41/78 (52.6%) of the comparisons among the bacteria, and in 5/6 (83.3%) of the comparisons among the yeasts. In Table 3, comparisons at 285-1100 nm with silicon dioxide are presented. It was possible to distinguish one microorganism from another in 109/136 (81.1%) of all the comparisons. There was a significant difference in 52/52 (100%) of the comparisons between yeasts and bacteria, 54/78 (69.2%) of the comparisons among bacteria, and in 3/6 (50%) of the comparisons among the yeasts.

### 3.2. Microorganisms compared at 285–700 nm

In Table 4, comparisons at 285-700 nm without silicon dioxide are presented. Distinguishing one microorganism from another was possible in 106/136 (77.9%) of all the comparisons. There was a significant difference in 44/52 (84.6%) of the comparisons between yeast and bacteria, in 56/78 (71.8%) of the comparisons among the bacteria, and in 6/6 (100%) of the comparisons among the yeasts.

Table 4. Comparison of microorganisms at 285–700 nm without silicon dioxide using the two-sample t-test.

| Compared Microorganisms                  | p value  |
|------------------------------------------|----------|
| Kluyveromyces marxianus compared to Candida albicans | 0.000034 |
| Kluyveromyces marxianus compared to Candida glabrata | <0.000001 |
| Kluyveromyces marxianus compared to Candida krusei | <0.000001 |
| Kluyveromyces marxianus compared to Staphylococcus epidermidis | 0.001229 |
| Kluyveromyces marxianus compared to Enterococcus faecalis | <0.000001 |
| Kluyveromyces marxianus compared to MSSA | 0.503270 |
| Kluyveromyces marxianus compared to MRSA | 0.925838 |
| Kluyveromyces marxianus compared to Bacillus spizizenii | <0.000001 |
| Kluyveromyces marxianus compared to Proteus mirabilis | <0.000001 |
| Kluyveromyces marxianus compared to Enterobacter aerogenes | <0.000001 |
| Candida albicans compared to Candida krusei | <0.000001 |
| Candida albicans compared to Staphylococcus epidermidis | <0.000001 |
| Candida albicans compared to Enterococcus faecalis | <0.000001 |
| Candida albicans compared to MSSA | 0.426952 |
| Candida albicans compared to MRSA | 0.135974 |
| Candida albicans compared to Bacillus spizizenii | <0.000001 |
| Candida albicans compared to Proteus mirabilis | <0.000001 |
| Candida albicans compared to Enterobacter aerogenes | <0.000001 |
| Candida albicans compared to Salmonella enteritidis | <0.000001 |
| Candida albicans compared to Pseudomonas aeruginosa | <0.000001 |
| Candida albicans compared to Citrobacter freundii | <0.000001 |
| Candida albicans compared to Klebsiella pneumoniae | <0.000001 |
| Candida albicans compared to Moraxella catarrhalis | <0.000001 |
| Candida albicans compared to Escherichia coli | <0.000001 |
| Candida glabrata compared to Candida krusei | <0.000001 |
| Candida glabrata compared to Staphylococcus epidermidis | <0.000001 |
| Candida glabrata compared to Enterococcus faecalis | <0.000001 |
| Candida glabrata compared to MSSA | 0.234312 |
| Candida glabrata compared to MRSA | 0.062722 |
| Candida glabrata compared to Bacillus spizizenii | <0.000001 |
| Candida glabrata compared to Proteus mirabilis | <0.000001 |
| Candida glabrata compared to Enterobacter aerogenes | <0.000001 |
| Candida glabrata compared to Salmonella enteritidis | <0.000001 |
| Candida glabrata compared to Pseudomonas aeruginosa | <0.000001 |
| Candida glabrata compared to Citrobacter freundii | <0.000001 |
| Candida glabrata compared to Klebsiella pneumoniae | <0.000001 |
| Candida glabrata compared to Moraxella catarrhalis | <0.000001 |
| Candida krusei compared to Staphylococcus epidermidis | 0.000004 |
| Candida krusei compared to Enterococcus faecalis | 0.006735 |
| Candida krusei compared to MSSA | <0.000001 |
| Candida krusei compared to MRSA | <0.000001 |
| Candida krusei compared to Bacillus spizizenii | <0.000001 |
| Candida krusei compared to Proteus mirabilis | 0.039461 |
| Candida krusei compared to Enterobacter aerogenes | 0.001872 |
| Candida krusei compared to Salmonella enteritidis | 0.003174 |
| Candida krusei compared to Pseudomonas aeruginosa | 0.519551 |
| Candida krusei compared to Citrobacter freundii | 0.031600 |
| Candida krusei compared to Klebsiella pneumoniae | <0.000001 |
| Candida krusei compared to Moraxella catarrhalis | 0.036041 |
| Candida krusei compared to Escherichia coli | 0.810323 |

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Table 4 (continued)

| Compared Microorganisms                                      | p value    |
|---------------------------------------------------------------|------------|
| Staphylococcus epidermidis compared to Enterococcus faecalis | <0.000001  |
| Staphylococcus epidermidis compared to MSSA                   | 0.007785   |
| Staphylococcus epidermidis compared to MRSA                   | 0.052664   |
| Staphylococcus epidermidis compared to Bacillus spizizenii    | <0.000001  |
| Staphylococcus epidermidis compared to Proteus mirabilis      | 0.000006   |
| Staphylococcus epidermidis compared to Enterobacter aerogenes | <0.000001  |
| Staphylococcus epidermidis compared to Salmonella enteritidis| <0.000001  |
| Staphylococcus epidermidis compared to Pseudomonas aeruginosa | 0.036588   |
| Staphylococcus epidermidis compared to Citrobacter freundii   | 0.000002   |
| Staphylococcus epidermidis compared to Klebsiella pneumoniae  | <0.000001  |
| Staphylococcus epidermidis compared to Moraxella catarrhals   | 0.139104   |
| Staphylococcus epidermidis compared to Escherichia coli       | 0.001456   |
| Enterococcus faecalis compared to MSSA                        | <0.000001  |
| Enterococcus faecalis compared to MRSA                        | <0.000001  |
| Enterococcus faecalis compared to Bacillus spizizenii         | <0.000001  |
| Enterococcus faecalis compared to Proteus mirabilis           | 0.708334   |
| Enterococcus faecalis compared to Enterobacter aerogenes      | 0.737493   |
| Enterococcus faecalis compared to Salmonella enteritidis      | 0.807431   |
| Enterococcus faecalis compared to Pseudomonas aeruginosa      | 0.021208   |
| Enterococcus faecalis compared to Citrobacter freundii        | 0.667577   |
| Enterococcus faecalis compared to Klebsiella pneumoniae       | <0.000001  |
| Enterococcus faecalis compared to Moraxella catarrhals        | 0.000360   |
| Enterococcus faecalis compared to Escherichia coli            | 0.067634   |
| MSSA compared to MRSA                                         | 0.581649   |
| MSSA compared to Bacillus spizizenii                          | <0.000001  |
| MSSA compared to Proteus mirabilis                           | <0.000001  |
| MSSA compared to Enterobacter aerogenes                       | <0.000001  |
| MSSA compared to Salmonella enteritidis                       | <0.000001  |
| MSSA compared to Pseudomonas aeruginosa                       | 0.000051   |
| MSSA compared to Citrobacter freundii                         | <0.000001  |
| MSSA compared to Klebsiella pneumoniae                        | <0.000001  |
| MSSA compared to Moraxella catarrhals                         | 0.000154   |
| MSSA compared to Escherichia coli                             | <0.000001  |
| MRSA compared to Bacillus spizizenii                          | <0.000001  |
| MRSA compared to Proteus mirabilis                            | <0.000001  |
| MRSA compared to Enterobacter aerogenes                       | <0.000001  |
| MRSA compared to Salmonella enteritidis                       | <0.000001  |
| MRSA compared to Pseudomonas aeruginosa                       | 0.000597   |
| MRSA compared to Citrobacter freundii                         | <0.000001  |
| MRSA compared to Klebsiella pneumoniae                        | <0.000001  |
| MRSA compared to Moraxella catarrhals                         | 0.002160   |
| MRSA compared to Escherichia coli                             | 0.000008   |
| Bacillus spizizenii compared to Proteus mirabilis              | <0.000001  |
| Bacillus spizizenii compared to Enterobacter aerogenes        | <0.000001  |
| Bacillus spizizenii compared to Salmonella enteritidis        | <0.000001  |
| Bacillus spizizenii compared to Pseudomonas aeruginosa        | <0.000001  |
| Bacillus spizizenii compared to Citrobacter freundii          | <0.000001  |
| Bacillus spizizenii compared to Klebsiella pneumoniae         | 0.396335   |
| Bacillus spizizenii compared to Moraxella catarrhals          | <0.000001  |
| Bacillus spizizenii compared to Escherichia coli              | <0.000001  |
| Proteus mirabilis compared to Enterobacter aerogenes          | 0.486847   |
| Proteus mirabilis compared to Salmonella enteritidis          | 0.545699   |
| Proteus mirabilis compared to Pseudomonas aeruginosa          | 0.675226   |
| Proteus mirabilis compared to Citrobacter freundii            | 0.968203   |
| Proteus mirabilis compared to Klebsiella pneumoniae           | <0.000001  |
| Proteus mirabilis compared to Moraxella catarrhals            | 0.002265   |
| Proteus mirabilis compared to Escherichia coli                | 0.164145   |
| Enterobacter aerogenes compared to Salmonella enteritidis     | 0.592737   |
| Enterobacter aerogenes compared to Pseudomonas aeruginosa     | 0.009600   |

Table 4 (continued)

| Compared Microorganisms                                      | p value    |
|---------------------------------------------------------------|------------|
| Enterobacter aerogenes compared to Citrobacter freundii       | 0.446085   |
| Enterobacter aerogenes compared to Klebsiella pneumoniae      | <0.000001  |
| Enterobacter aerogenes compared to Moraxella catarrhals       | 0.00106    |
| Enterobacter aerogenes compared to Escherichia coli          | 0.031823   |
| Salmonella enteritidis compared to Pseudomonas aeruginosa    | 0.012653   |
| Salmonella enteritidis compared to Citrobacter freundii      | 0.504783   |
| Salmonella enteritidis compared to Klebsiella pneumoniae     | <0.000001  |
| Salmonella enteritidis compared to Moraxella catarrhals      | 0.000174   |
| Salmonella enteritidis compared to Escherichia coli          | 0.014231   |
| Pseudomonas aeruginosa compared to Citrobacter freundii      | 0.054135   |
| Pseudomonas aeruginosa compared to Klebsiella pneumoniae     | <0.000001  |
| Pseudomonas aeruginosa compared to Moraxella catarrhals      | 0.411301   |
| Pseudomonas aeruginosa compared to Escherichia coli          | 0.506630   |
| Citrobacter freundii compared to Klebsiella pneumoniae       | <0.000001  |
| Citrobacter freundii compared to Moraxella catarrhals        | 0.001695   |
| Citrobacter freundii compared to Escherichia coli            | 0.160085   |
| Klebsiella pneumoniae compared to Moraxella catarrhals       | <0.000001  |
| Klebsiella pneumoniae compared to Escherichia coli           | <0.000001  |
| Moraxella catarrhals compared to Escherichia coli            | 0.090763   |

in 107/136 (78.7%) of all the comparisons. There was a significant difference in 44/52 (84.6%) of the comparisons between yeast and bacteria, in 58/78 (74.6%) of the comparisons among the bacteria, and in 5/6 (83.3%) of the comparisons among the yeasts.

3.3. Results summary

Results from the previous tables are summarized in Table 6. We found that silicon dioxide improved the probability of distinguishing one bacteria from another at wavelengths between 285–1100 nm. Silicon dioxide also decreased the probability of distinguishing one yeast from another. At 285–700 nm silicon dioxide did not have a significant effect on the results, but the probability of distinguishing one bacteria from another increased at this wavelength interval in comparison with the 285–1100 nm wavelength interval. Furthermore, the addition of silicon dioxide increased the ability to distinguish between Gram-positive and Gram-negative bacteria.

3.4. Silicon dioxide effects on average absorbance

Silicon dioxide decreased the average absorbance of most yeasts in the 285–1100 nm measurement interval, assuming a significant change of 0.03 absorbance units; and, it also increased the average absorbance of most bacteria in the same measurement interval (Table 7). For Candida glabrata, the average absorbance did not exhibit a significant decrease. For Staphylococcus epidermidis, the average absorbance after the addition of silicon dioxide decreased, but for Enterococcus faecalis, MSSA, Proteus mirabilis, and Escherichia coli silicon dioxide produced no significant changes.

3.5. Distinctive qualities of microorganism graphs

The average absorbance graphs of yeast, Gram-positive, and Gram-negative bacteria (Figure 1) showed distinctive characteristics corresponding to the type of microorganism the spectrophotometer was analyzing. The following graphs are shown from the longest wavelength to the shortest. This order was maintained as the spectrophotometer presented measured absorbance units starting at longer wavelengths. All of the yeast exhibited higher absorbance than the Gram-positive and Gram-negative bacteria at near infrared wavelengths. Absorbance for bacteria increased at a higher rate than the yeasts, and, at some point, the absorbance units of bacteria surpassed the absorbance units of the yeasts.
Table 5. Comparison of microorganisms at 285–700 nm with silicon dioxide using the two-sample t-test.

| Compared Microorganisms                          | p value   |
|-------------------------------------------------|-----------|
| Kluyveromyces marxianus compared to Candida albicans | 0.601437  |
| Kluyveromyces marxianus compared to Candida glabrata | <0.000001 |
| Kluyveromyces marxianus compared to Candida krusei  | <0.000001 |
| Kluyveromyces marxianus compared to Staphylococcus epidermidis | 0.993349  |
| Kluyveromyces marxianus compared to Enterococcus faecalis | <0.000001 |
| Kluyveromyces marxianus compared to MSSA           | 0.518039  |
| Kluyveromyces marxianus compared to MRSA          | <0.000001 |
| Kluyveromyces marxianus compared to Bacillus species | <0.000001 |
| Kluyveromyces marxianus compared to Proteus mirabilis | <0.000001 |
| Kluyveromyces marxianus compared to Enterobacter aerogenes | <0.000001 |
| Kluyveromyces marxianus compared to Salmoella enteritidis | <0.000001 |
| Kluyveromyces marxianus compared to Pseudomonas aeruginosa | <0.000001 |
| Kluyveromyces marxianus compared to Citrobacter freundii | <0.000001 |
| Kluyveromyces marxianus compared to Klebsiella pneumonia | 0.170752  |
| Kluyveromyces marxianus compared to Moraxella catarrhalis | <0.000001 |
| Candida albicans compared to Candida glabrata      | <0.000001 |
| Candida albicans compared to Candida krusei        | <0.000001 |
| Candida albicans compared to Staphylococcus epidermidis | 0.808098  |
| Candida albicans compared to Enterococcus faecalis | <0.000001 |
| Candida albicans compared to MSSA                   | 0.399741  |
| Candida albicans compared to MRSA                   | <0.000001 |
| Candida albicans compared to Bacillus species       | <0.000001 |
| Candida albicans compared to Proteus mirabilis      | <0.000001 |
| Candida albicans compared to Enterobacter aerogenes | <0.000001 |
| Candida albicans compared to Salmoella enteritidis  | <0.000001 |
| Candida albicans compared to Pseudomonas aeruginosa | <0.000001 |
| Candida albicans compared to Citrobacter freundii   | <0.000001 |
| Candida albicans compared to Klebsiella pneumonia    | 0.223873  |
| Candida albicans compared to Moraxella catarrhalis  | <0.000001 |
| Candida albicans compared to Escherichia coli       | <0.000001 |
| Candida glabrata compared to Candida krusei         | <0.000001 |
| Candida glabrata compared to Staphylococcus epidermidis | 0.000956  |
| Candida glabrata compared to Enterococcus faecalis  | <0.000001 |
| Candida glabrata compared to MSSA                    | 0.059364  |
| Candida glabrata compared to MRSA                   | 0.000168  |
| Candida glabrata compared to Bacillus species       | <0.000001 |
| Candida glabrata compared to Proteus mirabilis      | <0.000001 |
| Candida glabrata compared to Enterobacter aerogenes | <0.000001 |
| Candida glabrata compared to Salmoella enteritidis  | <0.000001 |
| Candida glabrata compared to Pseudomonas aeruginosa | <0.000001 |
| Candida glabrata compared to Citrobacter freundii   | <0.000001 |
| Candida glabrata compared to Klebsiella pneumonia    | 0.000314  |
| Candida glabrata compared to Moraxella catarrhalis  | <0.000001 |
| Candida glabrata compared to Escherichia coli       | <0.000001 |
| Candida krusei compared to Staphylococcus epidermidis | <0.000001 |
| Candida krusei compared to Enterococcus faecalis    | <0.000001 |
| Candida krusei compared to MSSA                      | <0.000001 |
| Candida krusei compared to MRSA                     | 0.169116  |
| Candida krusei compared to Bacillus species         | <0.000001 |
| Candida krusei compared to Proteus mirabilis        | <0.000001 |
| Candida krusei compared to Enterobacter aerogenes    | <0.000001 |
| Candida krusei compared to Salmoella enteritidis    | <0.000001 |
| Candida krusei compared to Pseudomonas aeruginosa    | 0.000310  |
| Candida krusei compared to Citrobacter freundii     | <0.000001 |
| Candida krusei compared to Klebsiella pneumonia      | <0.000001 |
| Candida krusei compared to Moraxella catarrhalis    | 0.000062  |
| Candida krusei compared to Escherichia coli         | <0.000001 |

(continued on next page)
Table 5 (continued)

| Compared Microorganisms | p value    |
|-------------------------|------------|
| Enterobacter aerogenes compared to Citrobacter freundii | 0.563406   |
| Enterobacter aerogenes compared to Klebsiella pneumoniae | <0.000001  |
| Enterobacter aerogenes compared to Moraxella catarrhalis | 0.000273   |
| Enterobacter aerogenes compared to Escherichia coli | 0.07978    |
| Salmonella enteritidis compared to Pseudomonas aeruginosa | 0.000025   |
| Salmonella enteritidis compared to Citrobacter freundii | 0.038555   |
| Salmonella enteritidis compared to Klebsiella pneumoniae | <0.000001  |
| Salmonella enteritidis compared to Moraxella catarrhalis | <0.000001  |
| Salmonella enteritidis compared to Escherichia coli | 0.00111    |
| Pseudomonas aeruginosa compared to Citrobacter freundii | 0.037290   |
| Pseudomonas aeruginosa compared to Klebsiella pneumoniae | <0.000001  |
| Pseudomonas aeruginosa compared to Moraxella catarrhalis | 0.705969   |
| Pseudomonas aeruginosa compared to Escherichia coli | 0.492234   |
| Citrobacter freundii compared to Klebsiella pneumoniae | <0.000001  |
| Citrobacter freundii compared to Moraxella catarrhalis | 0.001741   |
| Citrobacter freundii compared to Escherichia coli | 0.063245   |
| Klebsiella pneumoniae compared to Moraxella catarrhalis | <0.000001  |
| Klebsiella pneumoniae compared to Escherichia coli | <0.000001  |
| Moraxella catarrhalis compared to Escherichia coli | 0.230218   |

The increased rate of the bacteria was observed when examining the range of absorbance units for bacteria and yeast (Figure 2, Figure 3). However, at approximately 305 nm, all of the microorganisms displayed a dramatic spike in the increase of absorbance. This increase was more noticeable in the bacteria than the yeasts and may have been caused by the proteins and nucleic acids of the microorganisms [19]. Taken together, these criteria could be used to distinguish bacteria from yeast.

For Gram-negative bacteria, the spike occurring at the end of the measurement interval was, in comparison to Gram-positive bacteria, larger by an average of 0.303 absorbance units without silicon dioxide and by 0.345 absorbance units with silicon dioxide. This criterion might be used to distinguish Gram-negative bacteria from Gram-positive bacteria when using spectrophotometry, with the exception of the Gram-negative bacteria Klebsiella pneumonia and the Gram-positive bacteria Enterococcus faecalis. Klebsiella pneumonia appeared to have a lower spike, similar to Gram-positive bacteria, occurring at the end of the graph, while Enterococcus faecalis appeared to have a higher spike, similar to Gram-negative bacteria, also occurring at the end of the graph.

After the addition of silicon dioxide, the specific characteristics used to distinguish bacteria from yeasts without silicon dioxide remained the same (Figure 4). However, silicon dioxide did produce alterations in the microorganism graphs. Adding silicon dioxide decreased the average absorbance of all yeasts (Figure 5, Figure 6, Figure 7, Figure 8), with the exception Candida glabrata, which remained nearly the same (Figure 9, Figure 10). For all bacteria, absorbance increased at near infrared wavelengths. For several bacteria, absorbance increased in the visible light spectrum (380–700 nm) and for some, the increase occurred at ultraviolet (<380 nm) wavelengths (Figures 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, and 26, Table 8). Staphylococcus epidermidis exhibited the smallest increase in absorbance at near infrared wavelengths, which was barely noticeable from 1100 nm and decreased until 900 nm (Figure 12). Klebsiella pneumoniae had the most noticeable increase in absorbance after the addition of silicon dioxide, and its absorbance increased during the entire measurement interval (Figure 23). For Salmonella enteritidis, the increase in absorbance occurred during most of the measurement interval following the addition of silicon dioxide (Figure 20).

Table 6. Summary of all comparisons.

| Types of cultures compared | Comparisons with silicon dioxide at 285–1100 nm | Comparisons with silicon dioxide at 285–700 nm |
|----------------------------|-----------------------------------------------|-----------------------------------------------|
| Overall                    | 99/136 (72.8%)                                | 109/136 (81.1%)                               |
| Yeast compared to bacteria | 52/52 (100%)                                  | 44/52 (84.6%)                                 |
| Bacteria compared to bacteria (overall) | 41/78 (52.6%)                                               | 56/78 (71.8%)                                 |
| Gram-positive bacteria compared to Gram-negative bacteria | 28/40 (70.0%)                                              | 32/40 (80.0%)                                 |
| Yeast compared to yeast    | 5/6 (83.3%)                                   | 3/6 (50%)                                     |

Table 7. Effects of silicon dioxide on the average absorbance of different microorganisms.

| Microorganisms                               | Average absorbance without silicon dioxide | Average absorbance with silicon dioxide | Silicon dioxide effect on average absorbance |
|----------------------------------------------|--------------------------------------------|----------------------------------------|---------------------------------------------|
| Kluyveromyces marxianus                      | 1.262251                                   | 1.158944                               | Decrease                                    |
| Candida albicans                             | 1.227039                                   | 1.138811                               | Decrease                                    |
| Candida glabrata                             | 1.180000                                   | 1.168283                               | Decrease                                    |
| Candida kruse                                | 1.194647                                   | 1.134603                               | Decrease                                    |
| Staphylococcus epidermidis                   | 0.946494                                   | 0.895001                               | Decrease                                    |
| Enterococcus faecalis                       | 0.949259                                   | 0.942527                               | Did not alter                               |
| MSSA                                         | 0.793623                                   | 0.812563                               | Increase                                    |
| MRSA                                        | 0.830097                                   | 0.886495                               | Increase                                    |
| Bacillus subtilis                           | 0.715224                                   | 0.745946                               | Increase                                    |
| Proteus mirabilis                           | 0.935026                                   | 0.957992                               | Increase                                    |
| Enterobacter aerogenes                      | 0.938885                                   | 0.982013                               | Increase                                    |
| Salmonella enteritidis                      | 0.941685                                   | 1.006821                               | Increase                                    |
| Proteus mirabilis                           | 0.892040                                   | 0.930857                               | Increase                                    |
| Enterobacter aerogenes                      | 0.930903                                   | 0.991864                               | Increase                                    |
| Klebsiella pneumoniae                       | 0.661763                                   | 0.827743                               | Increase                                    |
| Moraxella catarrhalis                       | 0.897066                                   | 0.935586                               | Increase                                    |
| Escherichia coli                            | 0.900817                                   | 0.905401                               | Did not alter                               |
Figure 1. Average absorbance of yeast, Gram-positive and Gram-negative bacteria at 285–1100 nm without silicon dioxide.

Figure 2. Microorganism absorbance unit ranges without silicon dioxide. Kluyveromyces marxianus (A), Candida albicans (B), Candida glabrata (C), Candida krusei (D), Staphylococcus epidermidis (E), Enterococcus faecalis (F), MSSA (G), MRSA (H), Bacillus spizizenii (I), Proteus mirabilis (J), Enterobacter aerogenes (K), Salmonella enteritidis (L), Pseudomonas aeruginosa (M), Citrobacter freundii (N), Klebsiella pneumoniae (O), Moraxella catarrhalis (P), Escherichia coli (Q).
Figure 3. Microorganism absorbance unit ranges with silicon dioxide. *Kluyveromyces marxianus* (A), *Candida albicans* (B), *Candida glabrata* (C), *Candida krusei* (D), *Staphylococcus epidermidis* (E), *Enterococcus faecalis* (F), MSSA (G), MRSA (H), *Bacillus spizizenii* (I), *Proteus mirabilis* (J), *Enterobacter aerogenes* (K), *Salmonella enteritidis* (L), *Pseudomonas aeruginosa* (M), *Citrobacter freundii* (N), *Klebsiella pneumoniae* (O), *Moraxella catarrhalis* (P), *Escherichia coli* (Q).

Figure 4. Average absorbance of all examined yeasts, Gram-positive and Gram-negative bacteria at 285–1100 nm with silicon dioxide.
Figure 5. Average absorbance of all examined yeasts without and with silicon dioxide.

Figure 6. Absorbance of *Kluyveromyces marxianus* with and without silicon dioxide.
Figure 7. Absorbance of *Candida albicans* with and without silicon dioxide.

Figure 8. Absorbance of *Candida krusei* with and without silicon dioxide.
Figure 9. Absorbance of *Candida glabrata* with and without silicon dioxide.

Figure 10. Absorbance unit ranges for yeasts. *Kluyveromyces marxianus* (A1), *Kluyveromyces marxianus* with SiO$_2$ (A2), *Candida albicans* (B1), *Candida albicans* with SiO$_2$ (B2), *Candida glabrata* (C1), *Candida glabrata* with SiO$_2$ (C2), *Candida krusei* (D1), *Candida krusei* with SiO$_2$ (D2).
Figure 11. Average absorbance of all examined Gram-positive bacteria with and without silicon dioxide.

Figure 12. Absorbance of *Staphylococcus epidermidis* with and without silicon dioxide.
Figure 13. Absorbance of *Enterococcus faecalis* with and without silicon dioxide.

Figure 14. Absorbance of MSSA with and without silicon dioxide.
Figure 15. Absorbance of MRSA with and without silicon dioxide.

Figure 16. Absorbance of Bacillus spizizenii with and without silicon dioxide.
Figure 17. Average absorbance of all examined Gram-negative bacteria with and without silicon dioxide.

Figure 18. Absorbance of *Proteus mirabilis* with and without silicon dioxide.
Figure 19. Absorbance of Enterobacter aerogenes with and without silicon dioxide.

Figure 20. Absorbance of Salmonella enteritidis with and without silicon dioxide.
Figure 21. Absorbance of *Pseudomonas aeruginosa* with and without silicon dioxide.

Figure 22. Absorbance of *Citrobacter freundii* with and without silicon dioxide.
Figure 23. Absorbance of *Klebsiella pneumoniae* with and without silicon dioxide.

Figure 24. Absorbance of *Moraxella catarrhalis* with and without silicon dioxide.
Figure 25. Absorbance of *Escherichia coli* with and without silicon dioxide.

Figure 26. Absorbance unit ranges for bacteria. *Staphylococcus epidermidis* (A1), *Staphylococcus epidermidis* with SiO$_2$ (A2), *Enterococcus faecalis* (B1), *Enterococcus faecalis* with SiO$_2$ (B2), MSSA (C1), MSSA with SiO$_2$ (C2), MRSA (D1), MRSA with SiO$_2$ (D2), *Bacillus subtilis* (E1), *Bacillus subtilis* with SiO$_2$ (E2), *Proteus mirabilis* (F1), *Proteus mirabilis* with SiO$_2$ (F2), *Enterobacter aerogenes* (G1), *Enterobacter aerogenes* with SiO$_2$ (G2), *Salmonella enteritidis* (H1), *Salmonella enteritidis* with SiO$_2$ (H2), *Pseudomonas aeruginosa* (I1), *Pseudomonas aeruginosa* with SiO$_2$ (I2), *Citrobacter freundii* (J1), *Citrobacter freundii* with SiO$_2$ (J2), *Klebsiella pneumoniae* (K1), *Klebsiella pneumoniae* with SiO$_2$ (K2), *Moraxella catarrhalis* (L1), *Moraxella catarrhalis* with SiO$_2$ (L2), *Escherichia coli* (M1), *Escherichia coli* with SiO$_2$ (M2).
4. Discussion

4.1. Comparing yeast with bacteria

In this study, we demonstrated that spectrophotometry can be used to distinguish bacteria from yeasts at a wavelength interval of 285–1100 nm by measuring their absorbance.

Unpaired t-test's showed that adding silicon dioxide did not alter the probability of distinguishing bacteria from yeast at a wavelength interval of 285–1100 nm. A shorter wavelength interval of 285–700 nm also did not increase the likelihood of distinguishing bacteria from yeast, although by visually examining the graph it was possible to distinguish between the two. The most favourable conditions at which yeast-bacteria comparisons without silicon dioxide could be made occurred at 285–1100 nm. This suggests that a larger wavelength interval is best suited for comparing yeasts with bacteria.

4.2. Comparing bacteria to one another

Decreasing the wavelength interval from 285-1100 nm to 285–700 nm improved the likelihood of distinguishing bacteria from one another. At 285–1100 nm without silicon dioxide, only 52.6% of the bacterial-bacteria comparisons demonstrated a significant difference, but at 285–700 nm without silicon dioxide the results improved to 71.8%. A similar pattern occurred when silicon dioxide was added, as the results improved from 69.2% to 74.6%. This indicates that a smaller wavelength interval is best suited for comparing bacteria with each other. When
comparing Gram-positive to Gram-negative bacteria, the best results occurred at the 285–700 nm interval (without the need to take into account the addition of silicon dioxide). The probability of distinguishing between bacterial types with and without silicon dioxide was 80%.

4.3. Comparing yeasts to one another

The most favourable wavelengths at which yeast should be compared is between 285-700 nm with silicon dioxide, even though silicon dioxide did not provide a substantial light absorption change for yeast. At 285–700 nm without silicon dioxide it was possible to distinguish one yeast from another in 100% of the comparisons, but after the addition of silicon dioxide this possibility decreased to 83.3%; a 16.7% decrease. Likewise, at the measurement interval 285–1100 nm it was possible to distinguish one yeast from another in 83.3% of the comparisons, but when silicon dioxide was added the probability decreased to 50%; a 40% decrease. These results suggest that silicon dioxide should not be applied when comparing yeasts, and a wavelength of 285–700 nm is an appropriate interval for comparisons among yeasts as opposed to 285–1100 nm.

5. Conclusions

5.1. Potential practical applications

This study can provide a foundation for a new method of distinguishing microorganisms in a short period of time. To achieve this, there should be a database that contains each microorganism's absorbance within a specific substance and specific optical density.

This study may also provide the foundations for a new validation method for determining whether microorganisms bind to a specific substance, especially as fluorescence microscopy and electron microscopy, which are currently used to make these validations, can be time consuming.

5.2. Microorganism adhesion with silicon dioxide

Adding silicon dioxide increased the average absorbance of all examined bacteria at long wavelengths. The average absorbance of silicon dioxide is 0.211 absorbance units. The Beer-Lambert law states that a
mixture contains the combined absorbance of both samples, but this would only be the case if the concentration of each measured component is the same in the mixture as in pure samples [20]. If a suspension with high absorbance, such as bacteria or yeast, is mixed with a suspension with low absorbance and a sample is then taken, it is expected that the new absorbance would be less than that of the bacteria or yeast, as the mixed suspension would contain a smaller amount of bacteria or yeast per ml than the pure samples. However, for bacteria, this was not the case. The increase of absorbance at near infrared wavelengths, and for some bacteria at the visible light and ultraviolet wavelengths, indicates that bacteria made complexes with the silicon dioxide that exhibited an altered absorbance from the pure samples. A control measurement of *Escherichia coli* with fluorescence microscopy using a Leica TCS SL confocal microscope confirmed co-localization of *Escherichia coli* and silicon dioxide (Figure 27), while light microscopy with iodine confirmed *Escherichia coli* and *Enterococcus faecalis* adhesion to the silicon dioxide (Figure 28). For yeasts, however, the results were different. The absorbance did drop during the entire measurement for all of the yeasts, *Escherichia coli* con*fluent* microscope con*firm*ed that the absorbance was the same in the mixture as in pure samples [20]. If a suspension with a yeast and a bacterium contained the combined absorbance of both samples, but this new absorbance would be less than that of the bacteria or yeast, as the mixed suspension would contain a smaller amount of bacteria or yeast per ml than the pure samples. However, for bacteria, this was not the case. The increase of absorbance at near infrared wavelengths, and for some bacteria at the visible light and ultraviolet wavelengths, indicates that bacteria made complexes with the silicon dioxide that exhibited an altered absorbance from the pure samples. A control measurement of *Candida albicans* with fluorescence microscopy confirmed these suspicions as no noticeable co-localization was observed (Figure 29). *Candida albicans* adhesion with silicon dioxide was also not observed using light microscopy with iodine (Figure 28). This result is in accordance with another study claiming that silica nanoparticles reduces the attachment of *Candida albicans* to surfaces [21]. Our results suggest that bacteria have an adherence for silicon dioxide, and that it may be a poor choice of material for use in implantable prostheses. Our results also suggest that spectrophotometry might be used to confirm microorganism adhesion to inorganic surfaces as well as distinguish bacteria from yeast. For future studies we suggest using different inorganic materials to evaluate which types of microorganisms might adhere to them and whether such adhesions can be proven using spectrophotometry. Moreover, analysing one sample of each microorganism using fluorescent microscopy to confirm their adhesion to microparticles is suggested.

**Declarations**

**Author contribution statement**

Roberts Lozins: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Turs Selga: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Dzintars Ozoli: Conceived and designed the experiments; Wrote the paper.

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The authors declare no conflict of interest.

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