The Sterilization of Escherichia coli with Black Diamond-Coated Silicon

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Cover Page Footnote

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ABSTRACT

In order to combat increasing levels of antimicrobial resistance, new antimicrobials are needed to successfully kill microbes. Silicon coated in black diamond is a material that is hypothesized to have antimicrobial properties. To test this hypothesis, Escherichia coli cells were placed on different black diamond-coated silicon surfaces and allowed to rest on each surface for 15 minutes, 30 minutes, and 1 hour. Cells were collected, and growth was assessed by counting colonies on plates or spectrophotometry growth curves. The results of this study indicated that the experimental samples have some antimicrobial or growth inhibition properties, but they may not be to the extent as hypothesized. Errors in the harvesting method were likely present, and the experimental technique is currently being modified to collect the maximum number of cells for growth assessment.

KEYWORDS*: sterilization, black diamond, antimicrobial, antibacterial

INTRODUCTION

The development of new antimicrobial surfaces has the potential to reduce infectious outbreaks. As antimicrobial resistance continues to be an ongoing problem in preventing and treating diseases, the demand is increasing for new materials to effectively kill microbes. Transmission of infections can be reduced or even eliminated by coating commonly touched surfaces with these new materials (Tiller et al., 2001). Metallic copper has been the "gold standard" antimicrobial surface by killing microbes within minutes of contact through the membrane-damaging build-up of copper ions (Santo et al., 2011). However, copper is expensive to produce and has been shown to corrode upon contact with water (Szakalos et al., 2007).

Faculty of the University of Louisville Speed School of Engineering have developed a cheap and easy-to-produce material that may overcome this problem. This material is solid silicon coated in solid matte black diamond and is hypothesized to have antimicrobial properties. The nanostructured black-diamond coating was shown to kill bacteria by disrupting the cell membrane in a similar study (Hazell et al., 2018). Seven samples of black diamond-coated silicon were provided and tested for antimicrobial characteristics in this experiment using the model organism *Escherichia coli* (*E. coli*). There were no differences in the structures of the black diamond-coated samples. *E. coli* was used in this experiment because of its short generation time and few nutritional requirements (Taj et al., 2014). However, the results of this experiment may only be applicable to other gram-negative bacterial species because the mechanism of the surfaces' antimicrobial properties may be dependent on cell wall characteristics and organism motility (Hazell et al., 2018).

METHODS AND MATERIALS

Two different methods were utilized in this experiment. For the first method, one milliliter (mL) of stock *E. coli* strain DH5-α was inoculated in 20 mL of LB growth medium and incubated at 37°C for 24 hours with shaking to allow for sufficient growth. Five sample surfaces were tested in this experiment: copper, polyethylene, base silicon, sample 10, and sample 11. Copper was used as the positive control and polyethylene was the negative control because it has no known antimicrobial properties. Samples 10 and 11 were the experimental samples of diamond-coated silicon. Each sample was sterilized with 10% *v/v* bleach solution for ten seconds and rinsed with 1 mL of autoclaved deionized water three consecutive times. Each sample chip was gently dried with a Kimwipe. Sample test tubes were prepared with 0.5 mL of pure LB media in each. 5 μL directly from the *E. coli* culture were pipetted and spread on the surface of each sample chip. The *E. coli* was allowed to dry on each surface for 30 minutes. After 30 minutes, 1 mL of pure LB media was placed on each sample chip in the location of the *E. coli* cells and allowed to rest on the surface for 30 seconds. A pipette was used to collect the cells and the 1 mL of the LB media from each surface, and each sample was deposited in its respective test tube. With the addition of the 1 mL of LB media containing *E. coli* cells to the tubes with the initial 0.5 mL of pure LB media, a total of 1.5 mL of solution were in each test tube after collection.

Cells from each treatment were successfully transferred to the sample tubes, however, it was possible that the harvesting technique may have resulted in a minimal loss in the number of cells during the transfer from the surfaces to the test tubes.

A 1/10 dilution was completed by adding 0.1 mL of each sample into 0.9 mL of LB media. A 1/100 serial dilution was completed by pipetting 0.1 mL of each sample from the 1/10 dilution and adding it to a test tube that contained 0.9 mL.

Growth was assessed via viable cell plate counts. LB agar plates were prepared 24 hours prior to experimentation. In triplicate, 0.1 mL of each undiluted, 1/10 diluted, and 1/100 diluted sample were plated and spread onto the agar medium using an L-spreader. Pure *E. coli* strain DH5-α from the stock solution and its respective dilutions were plated for comparison. An *E. coli* control, which consisted of 5 µL of stock *E. coli* strain DH5-α added to 1.5mL of LB, was also plated with its respective dilutions to simulate what growth should look like if a surface did not kill any cells. Plates were incubated at 37ºC to allow for growth. Images were taken of the plates after 20.5 hours and 27 hours of incubation, and the surviving *E. coli* cells were counted as colonies. Another trial was completed using the same procedure 2 weeks later, but the cells were placed on each surface for 1 hour instead of 30 minutes. For this trial, the surviving *E. coli* cells were counted as colonies from images taken after 21 hours of incubation.

For the second method, the antimicrobial properties of sample 12, sample 15, sample 16, copper, polyethylene, and base silicon were tested. The following week, the same method was used to test sample 9, sample 5A, sample 16, copper, polyethylene, and base silicon. One milliliter of stock *E. coli* strain DH5-α was inoculated in 20 mL of LB growth medium and incubated at 37ºC for 24 hours to allow for sufficient growth. Each sample was sterilized with 10% *v/v* bleach solution for ten seconds and rinsed with 1 mL of autoclaved deionized water three consecutive times. Each sample chip was gently dried with a Kimwipe. Sample test tubes were prepared with 1.5 mL of LB media in each. 5 µL from the *E. coli* culture were pipetted on the surface of each sample chip. The *E. coli* was allowed to dry on each surface for 15 minutes. After 15 minutes, 30µL of sterile LB media were placed on each chip in the location of the *E. coli* cells and was allowed to rest for 30 seconds. The bacterial cells were collected with a sterile cotton swab by steaking to the right and left three times. Each surface was dry after streaking with the cotton swabs, so it was concluded that all cells were collected from each surface. The cotton swabs were placed in their respective test tubes that contained 1.5 mL of LB media for 1 minute to collect the

E. coli cells. This procedure was repeated two more times for a total of three trials.

Growth was assessed using a SpectraMax Spectrophotometer. 0.3 mL of each sample were added to each well of a 96-well plate in triplicate. Pure LB media was added to the first three wells of the plate for comparison. An *E. coli* control, which consisted of 5 µL of stock *E. coli* strain DH5-α added to 1.5mL of LB, was also added to simulate what growth should look like if a surface did not kill any cells. Absorbance readings were taken every 30 minutes for 20 hours at a wavelength of 600 nm and an incubation temperature of 37ºC with shaking. The absorbance data was used to create a growth curve for each sample in Microsoft Excel. An independent samples t-test was completed using the program GraphPad to test for a significant difference between the absorbance values for the *E. coli* control and each experimental sample.

RESULTS

For the 30-minute trial of the first experimental method, the plates with the 1/100 dilution had the best colony countability, so those plates were counted and compared between samples. The average number of colonies were reported per plate for the 1/100 dilution. The estimated concentration in colony forming units per milliliter (CFU/mL) and number of *E. coli* colony forming units (CFU) in the 1.5 mL tests tubes were calculated by the methods below. The dilution factor was 10^2 and the plating factor was 1/0.1 mL.

$$CFU/mL = (Number\ of\ Colonies) \times (Dilution\ Factor) \times (Plating\ Factor)$$

$$CFU = (CFU/mL) \times 1.5\ mL$$

For the count after 20.5 hours of incubation, the pure *E. coli* plates were completely saturated, and the *E. coli* control measured 112 colonies per plate. An estimated concentration of 1.12×10^5 CFU/mL and 6.80×10^5 CFU were present in the 1.5 mL *E. coli* control test tube. The base silicon had 53 colonies per plate, and an estimated concentration of 5.30×10^4 CFU/mL and 7.95×10^4 CFU were present in the test tube. The percent difference in the colony forming units between the *E. coli* control and base silicon was 88.3%. Copper had 3 colonies per plate and an estimated concentration of 3.00×10^3 CFU/mL and 4.50×10^3 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and copper was 99.3%. Sample 10 had 5 colonies per plate and an estimated concentration of 5.00×10^3 CFU/mL and

Sample	Colonies per Plate	Concentration in 1.5 mL Tube (CFU/mL)	CFU in 1.5 mL Tube	Percent Difference with *E. coli* Control
E. coli Control	112	1.12×10^5	6.80×10^5	--
Base Silicon	53	5.30×10^4	7.95×10^4	88.3%
Copper	3	3.00×10^3	4.50×10^3	99.3%
10	5	5.00×10^3	7.50×10^3	98.9%
11	1	1.00×10^3	1.50×10^3	99.8%
Polyethylene	6	6.00×10^3	9.00×10^3	98.7%

Table 1. *E. coli Growth on Plates After 27 Hours for the 1/100 Dilution*. The data for the number of colonies per plate, the estimated concentration and colony forming units in the 1.5 mL solution before plating, and percent difference with the E. coli control are displayed in this table for the 20.5-hour collection.
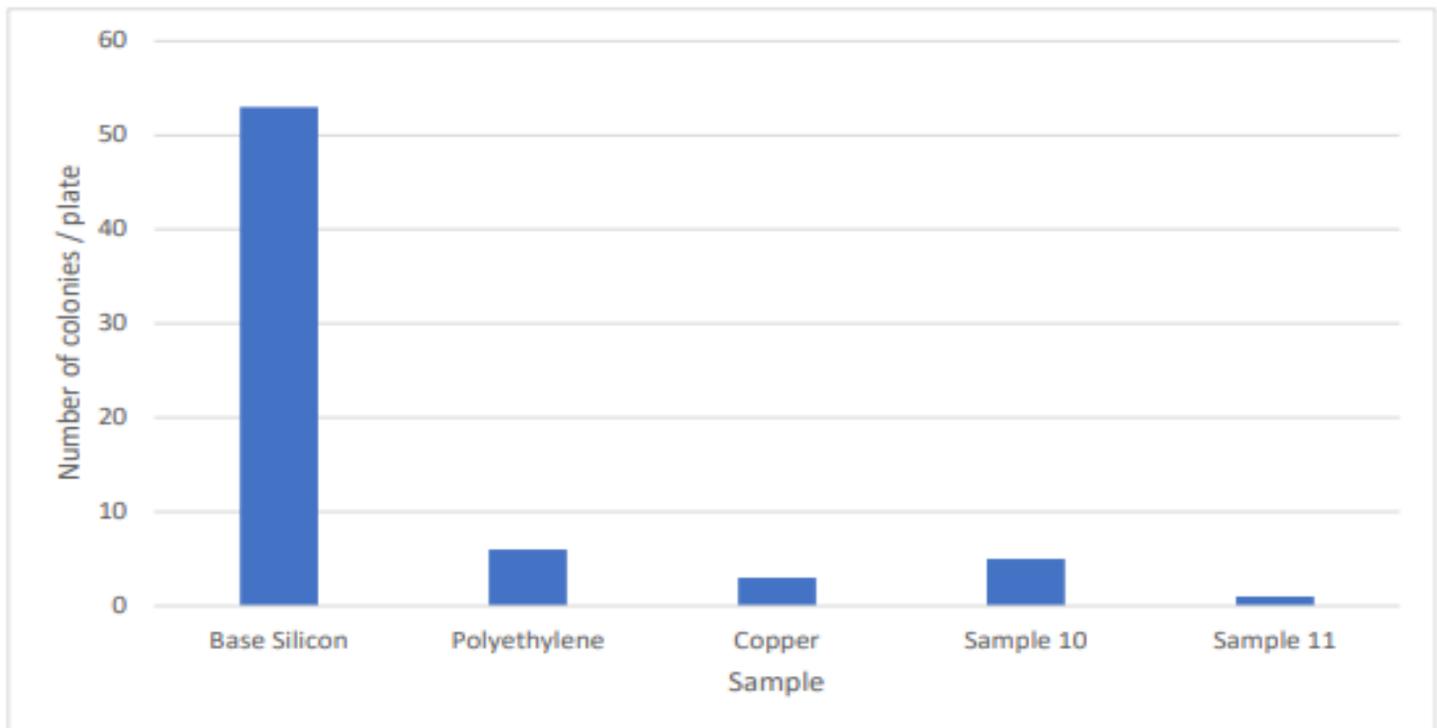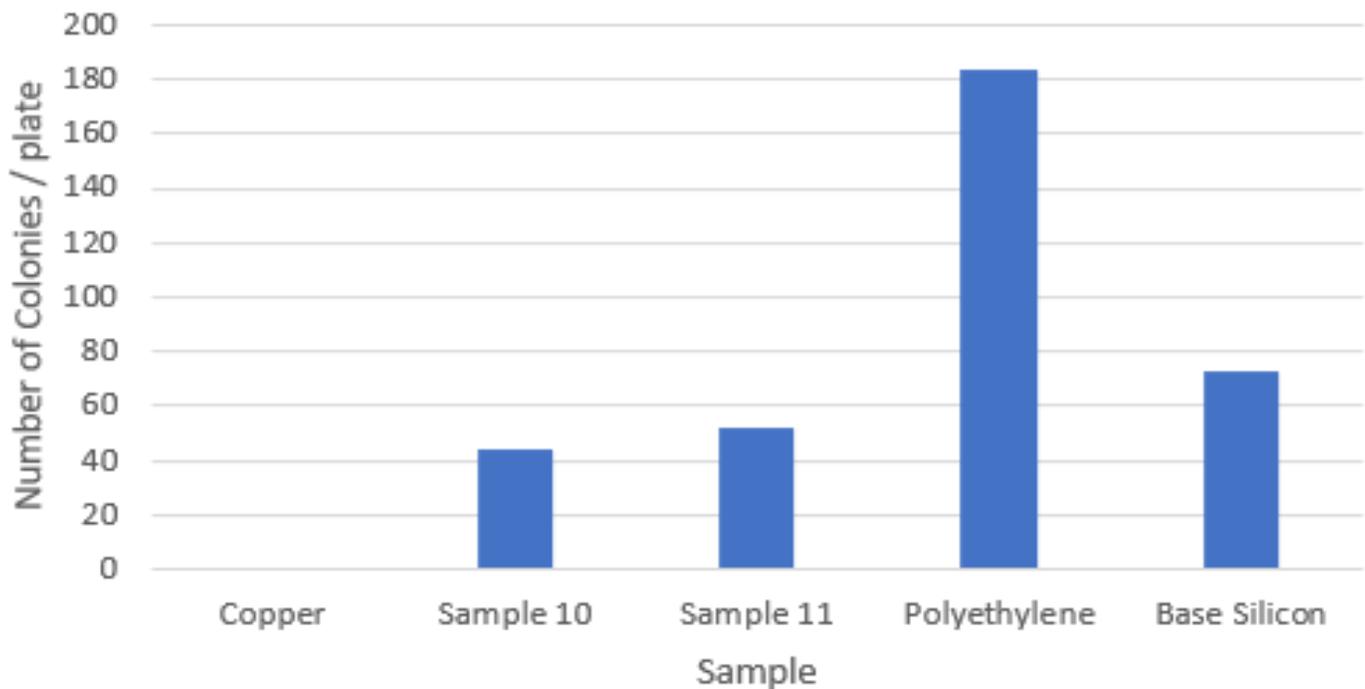

Figure 1. *Number of Colonies After 20.5 Hours for the 1/100 Dilution.* The number of colonies per plate for the 1/100 dilution after 20.5 hours of incubation are compared for base silicon, polyethylene, copper, sample 10, and sample 11. The E. coli control was excluded for scaling purposes.

7.50×10^3 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and sample 10 was 98.9%. Sample 11 measured 1 colony per plate and had an estimated concentration of 1.00×10^3 CFU/mL and 1.50×10^3 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and sample 11 was 99.8%. Polyethylene measured 6 colonies per plate and had an estimated concentration of 6.00×10^3 CFU/mL and 9.00×10^3 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and polyethylene was 98.7%. These results are depicted in Figure 1 and Table 1.

For the count after 27 hours of incubation, the pure *E. coli* samples were saturated, and the *E. coli* control measured 1813 colonies per plate. An estimated concentration of 1.81×10^6 CFU/mL and 2.72×10^6 CFU were present in the 1.5 mL *E. coli* control test tube. Copper had 1 colony per plate, and an estimated concentration of 1.00×10^3 CFU/mL and 1.50×10^3 CFU were present in the test tube. The percent difference in the colony forming units between the *E. coli* control and copper was 99.9%. Sample 10 had 44 colonies per plate and an estimated concentration of 4.40×10^4 CFU/mL and 6.60×10^4 CFU in the test tube. The percent difference in

Sample	Colonies per Plate	Concentration in 1.5 mL Tube (CFU/mL)	CFU in 1.5 mL Tube	Percent Difference with *E. coli* Control
E. coli Control	1813	1.81×10^6	2.72×10^6	--
Base Silicon	73	7.30×10^4	1.10×10^5	96.0%
Copper	1	1.00×10^3	1.50×10^3	99.9%
10	44	4.40×10^4	6.60×10^4	97.6%
11	52	5.20×10^4	7.80×10^4	97.1%
Polyethylene	184	1.84×10^5	2.76×10^5	89.9%

Table 2. *E. coli Growth on Plates After 27 Hours for the 1/100 Dilution*. The data for the number of colonies per plate, the estimated concentration and colony forming units in the 1.5 mL solution before plating, and percent difference with the E. coli control are displayed in this table for the 27-hour collection.

Figure 2. *Number of Colonies After 27 Hours for the 1/100 Dilution*. The number of colonies per plate for the 1/100 dilution after 27 hours of incubation for copper, sample 10, sample 11, polyethylene, and base silicon are compared in this figure. The E. coli control was excluded for scaling purposes.
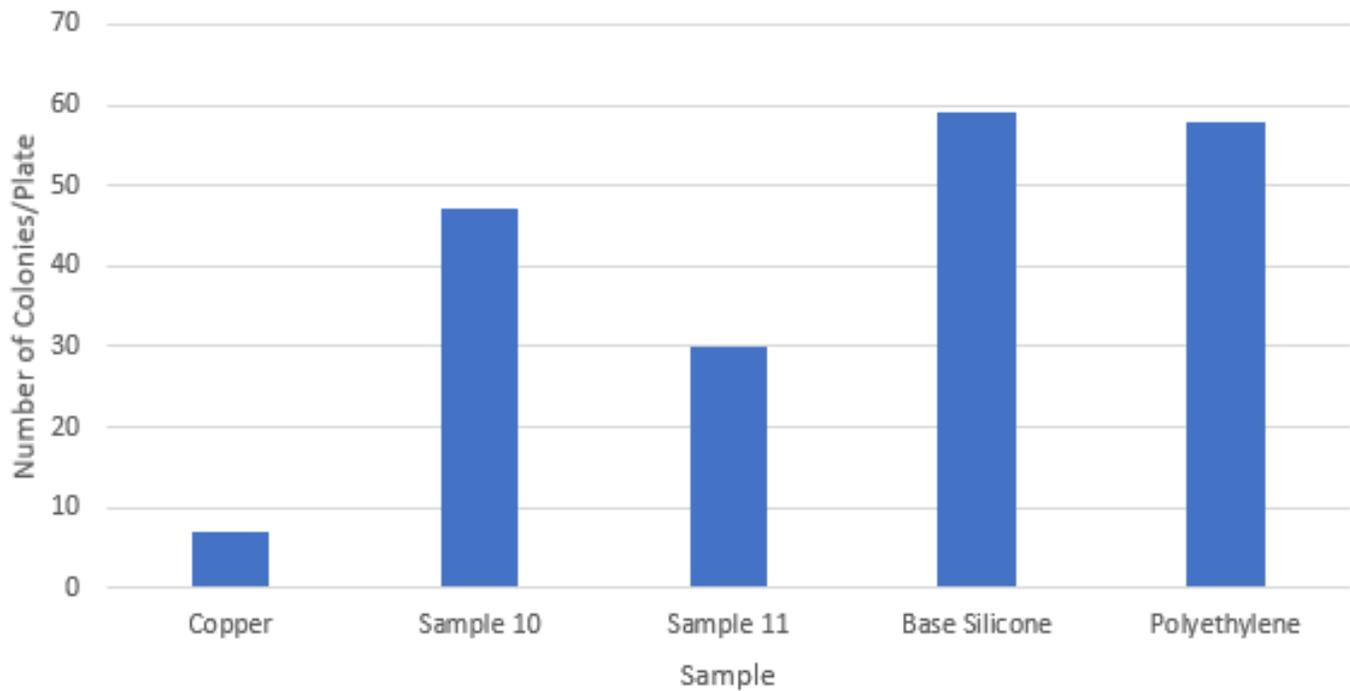
the colony forming units between the *E. coli* control and sample 10 was 97.6%. Sample 11 had 52 colonies per plate and an estimated concentration of 5.2×10^4 CFU/mL and 7.80×10^4 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and sample 11 was 97.1%. Base silicon measured 73 colony per plate and had an estimated concentration of 7.30×10^4 CFU/mL and 1.10×10^5 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and base silicon was 96.0%. Polyethylene measured 184 colonies per plate and had an estimated concentration of 1.84×10^5 CFU/mL and 2.76

$\times 10^5$ CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and polyethylene was 89.9%. These results are depicted in Figure 2 and Table 2.

For the 1-hour trial, the plates with the 1/100 dilution had the best colony countability, so those plates were counted and compared between samples after 21 hours of incubation. Some plates showed a small amount of contamination on the edge of the plate, so the average number of colonies were reported per plate for the 1/100 dilution. The pure *E. coli* samples were completely saturated, and the *E. coli* control measured 1,603 colonies

| Sample	Colonies per Plate	Concentration in 1.5 mL Tube (CFU/mL)	CFU in 1.5 mL Tube	Percent Difference with *E. coli* Control
E. coli Control	1603	1.60×10^6	2.40×10^6	--
Base Silicon	59	5.90×10^4	8.85×10^4	96.3%
Copper	7	7.00×10^3	1.10×10^4	99.5%
10	46	4.60×10^4	6.90×10^4	97.1%
11	30	3.00×10^4	4.50×10^4	98.1%
Polyethylene	58	5.80×10^4	8.70×10^4	96.3%

Table 3. *E. coli Growth on Plates After 21 Hours for the 1/100 Dilution*. The data for the number of colonies per plate, the estimated concentration and colony forming units in the 1.5 mL solution before plating, and percent difference with the E. coli control are displayed in this table for the 21-hour collection.

Figure 3. *Number of Colonies After 21 Hours for the 1/100 Dilution*. The number of colonies per plate for the 1/100 dilution after 21 hours of incubation are compared for copper, sample 10, sample 11, base silicon, and polyethylene. The E. coli control was excluded for scaling purposes.

per plate. An estimated concentration of 1.60×10^6 CFU/mL and 2.40×10^6 CFU were present in the 1.5 mL *E. coli* control test tube. Copper had 7 colonies per plate, and an estimated concentration of 7.00×10^3 CFU/mL and 1.10×10^4 CFU were present in the test tube. The percent difference in the colony forming units between the *E. coli* control and copper was 99.5%. Sample 10 had 46 colonies per plate and an estimated concentration of 4.46×10^4 CFU/mL and 6.90×10^4 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and sample 10 was 97.1%. Sample 11 had 30 colonies per plate and an estimated concentration of 3.00×10^4 CFU/mL and 4.50×10^4 CFU in the test tube. The

percent difference in the colony forming units between the *E. coli* control and sample 11 was 98.1%. Base silicon measured 59 colony per plate and had an estimated concentration of 5.90×10^4 CFU/mL and 8.85×10^4 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and base silicon was 96.3%. Polyethylene measured 58 colonies per plate and had an estimated concentration of 5.80×10^4 CFU/mL and 8.70×10^4 CFU in the test tube. The percent difference in the colony forming units between the *E. coli* control and polyethylene was 96.3%. These results are displayed in Figure 3 and Table 3.
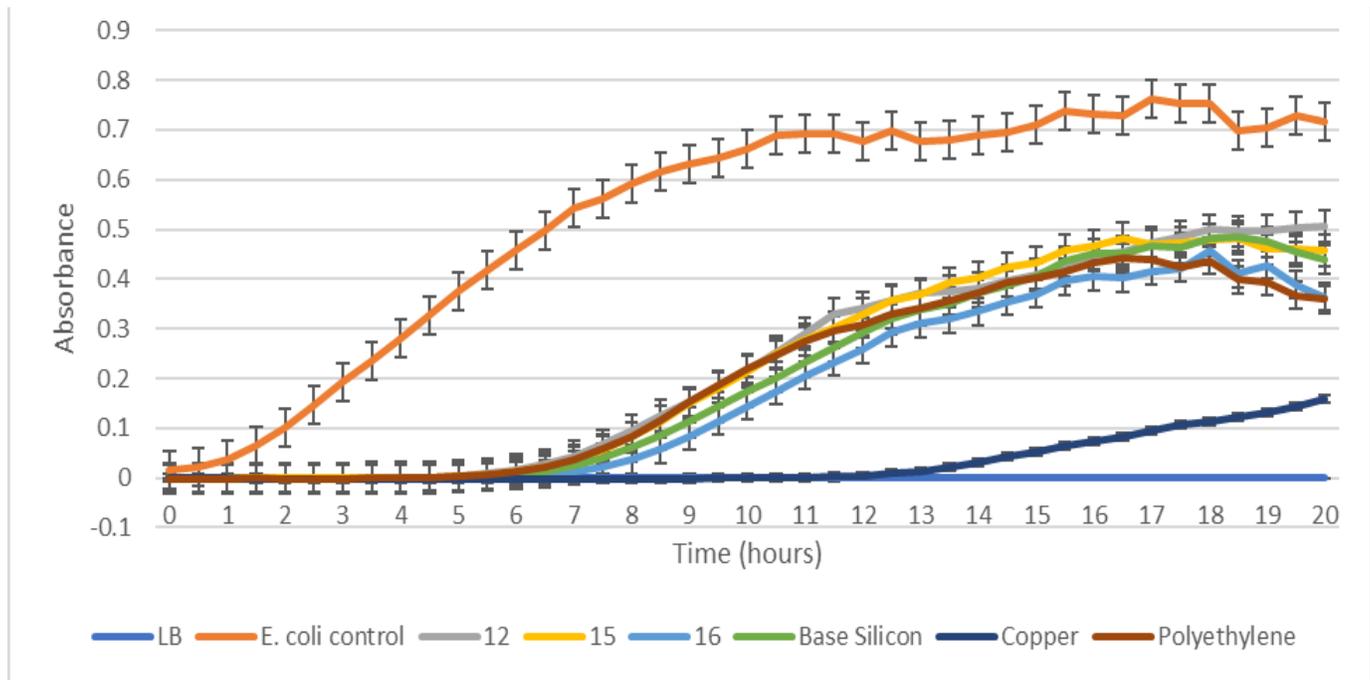
Figure 4. *E. coli Growth Curve*. The E. coli growth curves for the E. coli control, sample 12, sample 15, sample 16, base silicon, copper, and polyethylene are displayed in the figure. Error bars represent the standard deviation at each hour.

Sample	Absorbance 8 hours	SD 8 Hours	t-Test Value	P Value
E. coli control	0.590	0.045	--	--
12	0.096	0.072	10.0774	p < 0.05
15	0.082	0.041	14.4535	p < 0.05
16	0.037	0.022	19.1221	p < 0.05
Base silicon	0.062	0.055	12.8691	p < 0.05
Copper	-0.001	0.001	22.7420	p < 0.05
Polyethylene	0.084	0.043	14.0809	p < 0.05

Table 4. *Absorbance Data for E. coli After 8 Hours*. The absorbance values with respective standard deviations, t-test values, and p values at 8 hours for the E. coli control, sample 12, sample 15, sample 16, base silicon, copper, and polyethylene are displayed in this table.

For the second experimental method, the growth curves for *E. coli* on sample 12, sample 15, sample 16, copper, polyethylene, and base silicon are displayed in Figure 4. The growth curves do not differentiate until hour 8. At hour 8, the *E. coli* control had an average absorbance value among the three trials of 0.590 ($SD = 0.045$). Sample 12 had an average absorbance of 0.096 ($SD = 0.072$), and this value was significantly different from the *E. coli* control (t(4) = 10.0774, p < 0.05). Sample 15 had an average value of 0.082 ($SD = 0.041$), and this value was significantly different from the *E. coli* control (t(4) = 14.4535, p < 0.05). Sample 16 had an average of 0.037 ($SD = 0.022$), and this value was significantly different from the *E. coli* control (t(4) = 19.1221, p < 0.05). Base silicon had an average absorbance value of 0.062 ($SD = 0.055$), and this value was significantly different from the *E. coli* control (t(4) = 12.8691, p < 0.05). Copper had an average value of -0.001 ($SD = 0.001$), and this value was significantly different from the *E. coli* control (t(4) = 22.742, p < 0.05). Polyethylene had an average of 0.084 ($SD = 0.043$), and this value was also significantly different from the *E. coli* control (t(4) = 14.0809, p < 0.05). These data are summarized in Table 4.

Sample	Absorbance 11 hours	*SD* 11 Hours	T-Test Value	P-Value
E. coli control	0.692	0.101	--	--
12	0.290	0.144	3.9587	$p < 0.05$
15	0.277	0.068	5.9035	$p < 0.05$
16	0.205	0.052	7.4252	$p < 0.05$
Base silicon	0.233	0.134	4.7378	$p < 0.05$
Copper	0.001	0.002	11.8476	$p < 0.05$
Polyethylene	0.274	0.087	5.4312	$p < 0.05$

Table 5. *Absorbance Data for E. coli After 11 Hours.* The absorbance values with respective standard deviations, t-test values, and p values at 11 hours for the E. coli control, sample 12, sample 15, sample 16, base silicon, copper, and polyethylene are displayed in this table.

Sample	Absorbance 15 hours	*SD* 15 Hours	T-Test Value	P Value
E. coli control	0.712	0.133	--	--
12	0.410	0.118	2.9419	$p < 0.05$
15	0.434	0.085	3.0506	$p < 0.05$
16	0.370	0.023	4.3887	$p < 0.05$
Base silicon	0.406	0.131	2.8391	$p < 0.05$
Copper	0.053	0.088	7.1573	$p < 0.05$
Polyethylene	0.403	0.049	3.7760	$p < 0.05$

Table 6. *Absorbance Data for E. coli After 15 Hours.* The absorbance values with respective standard deviations, t-test values, and p values at 15 hours for the E. coli control, sample 12, sample 15, sample 16, base silicon, copper, and polyethylene are displayed in this table.

At hour 11, the *E. coli* control had an average absorbance value among the three trials of 0.692 ($SD = 0.101$). Sample 12 had an average absorbance of 0.290 ($SD = 0.144$), and this value was significantly different from the *E. coli* control ($t(4) = 3.9587$, $p < 0.05$). Sample 15 had an average value of 0.277 ($SD = 0.068$), and this difference with the *E. coli* control was significant ($t(4) = 5.9035$, $p < 0.05$). Sample 16 had an average of 0.205 ($SD = 0.052$), and this difference with the *E. coli* control was significant ($t(4) = 7.4252$, $p < 0.05$). Base silicon had an average absorbance value of 0.233 ($SD = 0.134$), and this difference with the *E. coli* control was significant ($t(4) = 4.7378$, $p < 0.05$). Copper had an average value of 0.001 ($SD = 0.002$), and this difference with the *E. coli* control was significant ($t(4) = 11.8476$, $p < 0.05$). Polyethylene had an average of 0.274 ($SD = 0.087$), and this difference with the *E. coli* control was significant ($t(4) = 5.4312$, $p < 0.05$). These data are summarized in Table 5.

At hour 15, the *E. coli* control had an average absorbance value among the three trials of 0.712 ($SD = 0.133$). Sample 12 had an average absorbance of 0.410 ($SD = 0.118$), and this value was significantly different from the *E. coli* control ($t(4) = 2.9419$, $p < 0.05$). Sample 15 had an average value of 0.434 ($SD = 0.085$), and this value was significantly different from the *E. coli* control ($t(4) = 3.0506$, $p < 0.05$). Sample 16 had an average of 0.370 ($SD = 0.023$), and this value was significant ($t(4) = 4.3887$, $p < 0.05$). Base silicon had an average absorbance value of 0.406 ($SD = 0.131$), and this value was significant ($t(4) = 2.8391$, $p < 0.05$). Copper had an average value of 0.053 ($SD = 0.088$), and this value was significant ($t(4) = 7.1573$, $p < 0.05$). Polyethylene had an average of 0.403 ($SD = 0.049$), and this value was significant ($t(4) = 3.776$, $p < 0.05$). These data are summarized in Table 6.

The growth curves for *E. coli* on sample 5A, sample 9, sample 16, copper, polyethylene, and base silicon are
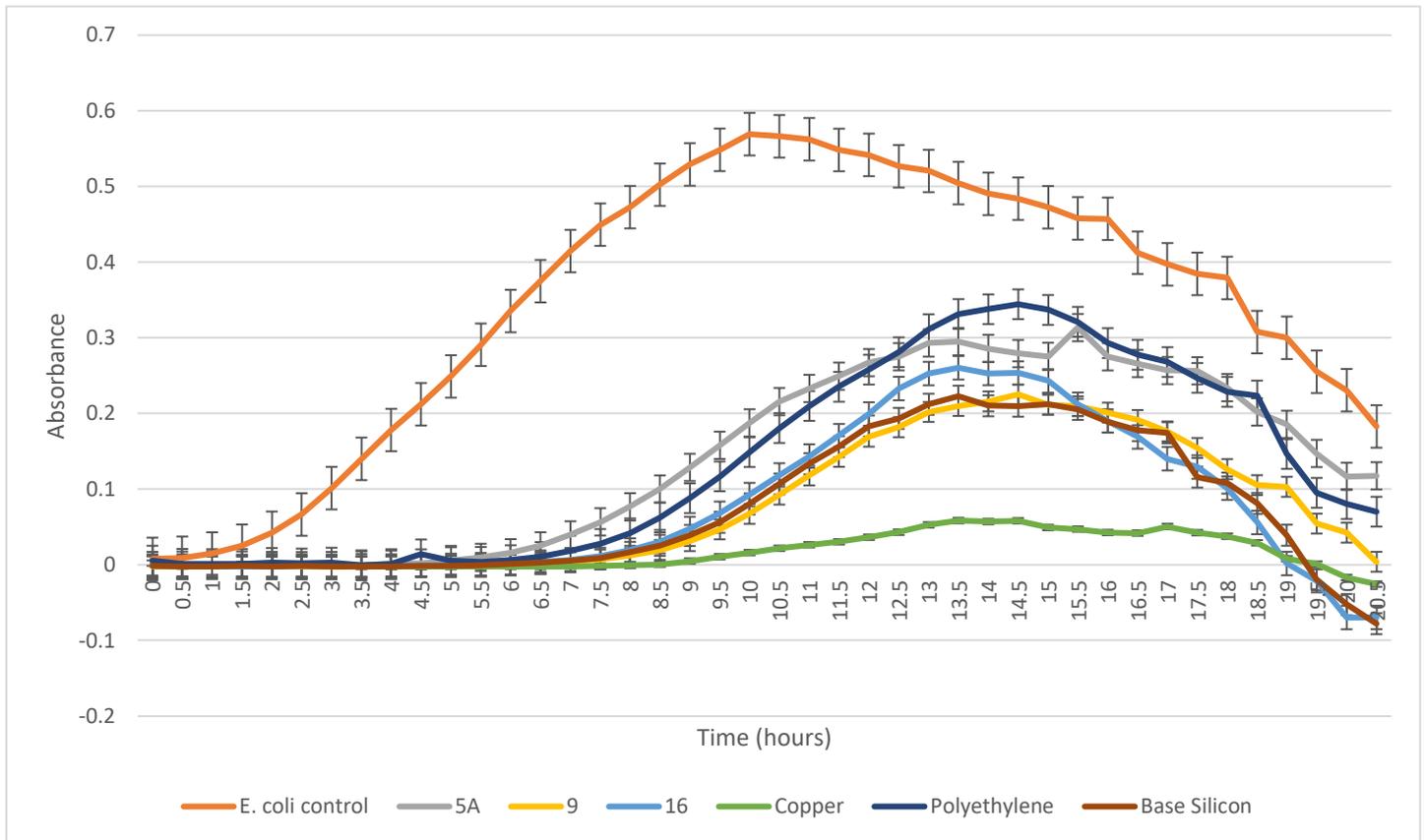
Figure 5. *E. coli Growth Curve.* The E. coli growth curve for E. coli control, sample 5A, sample 9, sample 16, base silicon, copper, and polyethylene are displayed in this figure. The error bars represent standard deviation at each hour.

Sample	Absorbance 8 hours	SD 8 Hours	T-test Value	P value
E. coli control	0.473	0.057	--	--
5A	0.077	0.070	7.5981	p < 0.05
9	0.012	0.010	13.7976	p < 0.05
16	0.019	0.013	13.4503	p < 0.05
Base silicon	0.017	0.004	13.8224	p < 0.05
Copper	-0.001	0.003	14.3835	p < 0.05
Polyethylene	0.042	0.033	11.3343	p < 0.05

Table 7. *Absorbance Data for E. coli Growth at 8 Hours.* The absorbance values with respective standard deviations, t-test values, and p values at 8 hours for the E. coli control, sample 5A, sample 9, sample 16, base silicon, copper, and polyethylene are displayed in this figure.

shown in Figure 5. Again, clear differentiation between growth curves cannot be observed until hour 8. At hour 8, the *E. coli* control had an average absorbance value among the three trials of 0.473 ($SD = 0.057$). Sample 5A had an average absorbance of 0.077 ($SD = 0.070$), and this was significantly different from the *E. coli* control (t(4) = 7.5981, p < 0.05). Sample 9 had an average absorbance of 0.012 ($SD = 0.010$), and this value was significantly different from the *E. coli* control (t(4) = 13.7976, p <

0.05). Sample 16 had an average of 0.019 ($SD = 0.013$), and this value compared with the *E. coli* control was significant (t(4) = 13.4503, p < 0.05). Copper had an average value of -0.001 ($SD = 0.003$), and this value was significantly different from the E. coli control (t(4) = 14.3835, p < 0.05). Polyethylene had an average value of 0.042 ($SD = 0.033$), and this value was significantly different from the E. coli control (t(4) = 11.3343, p < 0.05). Base silicon had an average value of 0.017 ($SD = 0.004$),

Sample	Absorbance 11 hours	SD 11 Hours	T-test Value	P Value
E. coli control	0.562	0.091	--	--
5A	0.233	0.084	4.6014	p < 0.05
9	0.118	0.038	7.7983	p < 0.05
16	0.144	0.051	6.9404	p < 0.05
Base silicon	0.134	0.026	7.8329	p < 0.05
Copper	0.030	0.040	9.2698	p < 0.05
Polyethylene	0.210	0.117	4.1133	p < 0.05

Table 8. *Absorbance Data for E. coli Growth at 11 Hours.* The absorbance values with respective standard deviations, t-test values, and p values at 11 hours for the E. coli control, sample 5A, sample 9, sample 16, base silicon, copper, and polyethylene are displayed in this figure.

Sample	Absorbance 15 hours	SD 15 Hours	T-Test Value	P Value
E. coli control	0.473	0.073	--	--
5A	0.275	0.065	3.5086	p < 0.05
9	0.212	0.032	5.6170	p < 0.05
16	0.243	0.068	3.9931	p < 0.05
Base silicon	0.213	0.058	4.8300	p < 0.05
Copper	0.049	0.084	6.5990	p < 0.05
Polyethylene	0.337	0.197	1.1212	p > 0.05

Table 9. *Absorbance Data for E. coli Growth at 15 Hours.* The absorbance values with respective standard deviations, t-test values, and p values at 15 hours for the E. coli control, sample 5A, sample 9, sample 16, base silicon, copper, and polyethylene are displayed in this figure.

and this value was also significantly different (t(4) = 13.8224, p < 0.05). These data are summarized in Table 7.

At hour 11, the *E. coli* control had an average absorbance value among the three trials of 0.562 (*SD* = 0.091). Sample 5A had an average absorbance of 0.233 (*SD* = 0.084), and this was significantly different from the *E. coli* control (t(4) = 4.6014, p < 0.05). Sample 9 had an average absorbance of 0.118 (*SD* = 0.038), and this value was significantly different from the *E. coli* control (t(4) = 7.7983, p < 0.05). Sample 16 had an average of 0.144 (*SD* = 0.051), and this value compared with the *E. coli* control was significant (t(4) = 6.9404, p < 0.05). Copper had an average value of 0.030 (*SD* = 0.040), and this value was significantly different from the E. coli control (t(4) = 9.2698, p < 0.05). Polyethylene had an average value of 0.210 (*SD* = 0.117), and this value was significantly

different from the E. coli control (t(4) = 4.1133, p < 0.05). Base silicon had an average value of 0.134 (*SD* = 0.026), and this value was also significantly different (t(4) = 7.8329, p < 0.05). These data are summarized in Table 8.

At hour 15, the *E. coli* control had an average absorbance value among the three trials of 0.473 (*SD* = 0.073). Sample 5A had an average absorbance of 0.275 (*SD* = 0.065), and this was significantly different from the *E. coli* control (t(4) = 3.5086, p < 0.05). Sample 9 had an average absorbance of 0.212 (*SD* = 0.032), and this value was significantly different from the *E. coli* control (t(4) = 5.617, p < 0.05). Sample 16 had an average of 0.243 (*SD* = 0.068), and this value compared with the *E. coli* control was significant (t(4) = 3.9931, p < 0.05). Copper had an average value of 0.049 (*SD* = 0.084), and this value was significantly different from the E. coli control (t(4) = 6.599, p < 0.05). Polyethylene had an average value of

0.337 (*SD* = 0.197), and this value was not significantly different from the E. coli control (t(4) = 1.1212, p > 0.05. Base silicon had an average value of 0.213 (*SD* = 0.058), and this value was significantly different (t(4) = 4.83, p < 0.05). These data are summarized in Table 9.

DISCUSSION

For the 30-minute trial of the first experimental procedure, all samples showed reduced growth compared to the *E. coli* control after 20.5 hours of incubation on plates. All samples also had a relatively large percent difference when compared to the *E. coli* control. Samples 10 and 11 had a similar number of colonies compared to copper, which demonstrated that some antimicrobial properties are present in those samples. These results are consistent with a study conducted by Dunseath et al., which concluded black diamond nanostructures significantly reduced the number of viable *E.coli* cells on the fabricated surface (Dunseath et al., 2019). The base silicon and polyethylene samples had the smallest percent difference values when compared to the *E. coli* control. These samples were not expected to have antimicrobial activity, but reduced growth was seen in both samples. This could have been caused by human experimental error involving the harvesting of the cells. It was possible that every cell was not transferred from the samples to the test tubes, which would have reduced the number of cells counted on the plates after incubation. This potential problem may have caused the polyethylene and base silicon samples to appear to have reduced the growth of the *E. coli* when they actually did not. Another source of error could have been from the colony count estimations. There was a greater possibility of inaccurate counts for the high-colony number plates, and the zoomed-in images of the plates were slightly blurry, which could have led to an incorrect number of colonies counted.

After 27 hours of incubation on plates for the same trial, polyethylene and base silicon both had more colonies than copper, sample 10, and sample 11, and these results were expected. Both the polyethylene and base silicon samples had the two smallest percent difference values when compared to the *E. coli* control. Copper had the greatest percent difference, and samples 10 and 11 also had relatively large percent difference values. Samples 10 and 11 showed more growth than copper, but fewer colonies grew compared to the *E. coli* control. This demonstrated that some antimicrobial activity or growth inhibition was present in the experimental samples, but not to the extent of copper.

For the 1-hour trial, all samples showed reduced growth compared to the *E. coli* control after 21 hours of incubation on plates. The polyethylene and base silicon samples had more *E. coli* growth compared to copper, sample 10, and sample 11. Sample 11 showed less growth compared to sample 10, but both samples had more colonies than copper. Again, the base silicon and polyethylene had the two smallest percent difference values, copper had the largest, and samples 10 and 11 also had a relatively large percent difference values when compared with the *E. coli* control. Samples 10 and 11 both showed reduced growth, but not to the same degree as copper. These results again represent some antimicrobial activity for the experimental samples, but this inhibition is not as strong as copper's.

For the second experimental method analyzed with spectrophotometry, a clear differentiation in growth curves can be seen for the samples beyond the 8-hour time point. This later differentiation demonstrated that the antimicrobial properties of the surfaces inhibited growth for approximately 8 hours. The effectiveness of each sample was compared through the differentiation of the growth curves. The *E. coli* control had the most rapid growth and highest absorbance values at all timepoints. The growth curves for sample 12, sample 15, sample 16, base silicon, and polyethylene were all clustered at similar absorbance readings between the *E. coli* control and copper for each timepoint. The growth curve for copper was lower than all samples at each timepoint. The average absorbance values for all samples at all measured time points were significantly different from the *E. coli* control. These results indicate antimicrobial activity or growth inhibition of sample 12, sample 15, sample 16, base silicon, and polyethylene. Base silicon and polyethylene were not expected to show reduced growth or to be significantly different from the *E. coli* control, so the harvesting method may have been a source of error. It was possible that when cells were harvested for growth analysis, all of the living cells were not collected, which would result in a smaller growth curve compared to the *E. coli* control.

When sample 5A, sample 9, sample 16, copper, polyethylene, and base silicon were tested with the second experimental method and analyzed with spectrophotometry, the *E. coli* control showed the most rapid growth curve and highest absorbance readings for all timepoints. Copper clearly showed the smallest growth curve, which was expected for the positive control. The absorbance reading for polyethylene at the 15-hour time point was significantly different from the *E. coli* control, which was also expected for negative control. Sample 5A, sample 9, sample 16, polyethylene, and base silicon all showed growth curves between the *E. coli* control and copper. Polyethylene and base silicon were not hypothesized to have antimicrobial activity, so the growth curves for these two samples should have been similar to the *E. coli* control, but they were instead closer to the curves of our experimental samples. The again indicates that errors may be present in the harvesting technique.

Samples 5A, 9, and 16 showed reduced growth curves and were significantly different compared to the control, which demonstrates that the surfaces are to some extent killing or inhibiting growth of the bacteria. However, the growth levels were still higher than those of copper, which suggests that the antimicrobial properties of the experimental samples are not as strong as hypothesized

CONCLUSIONS

When data was analyzed across all samples and experimental methods, samples 5A, 9, 10, 11, 12, 15, and 16 likely had some antimicrobial activity or growth inhibition properties. More *E. coli* cells grew in all of the experimental samples tested when compared to copper, the "gold standard". These preliminary results indicate that the antimicrobial pathways of the experimental samples were not as effective as those present in copper. Because polyethylene and base silicon showed reduced growth when antimicrobial properties were not expected for those surfaces, errors may have been present in the harvesting technique. Further testing and manipulation of the experimental methods are needed to assess the level of antimicrobial activity and growth inhibition of the experimental samples. Currently, the harvesting technique is being modified to collect the maximum amount of *E. coli* cells. Additional tests are also planned to assess growth after 24 hours of *E. coli* cell contact with each sample.

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