

Porous Antimicrobial Coatings for Killing Microbes within Minutes

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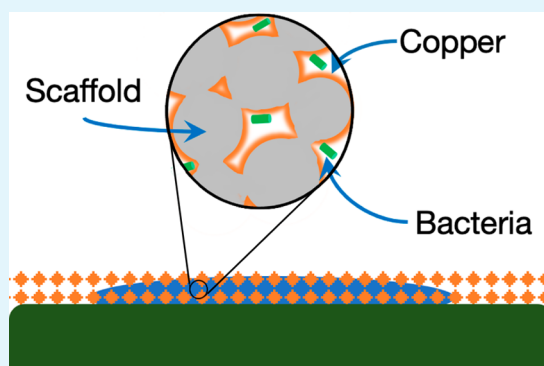
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ABSTRACT: Antimicrobial coatings can be used to reduce the transmission of infectious agents that are spread by contact. An effective coating should kill microbes in the time between users, which is sometimes minutes or less. Fast killing requires fast transport, and our proposed method of fast transport is a porous coating where the contaminated liquid imbibes (infiltrates) into the pores to achieve rapid contact with active material inside the pores. We test the hypothesis that a porous antimicrobial coating will enable faster inactivation of microorganisms than a planar coating of the same material. We use hydrophilic pores with dimensions of 5–100 μm such that liquid droplets imbibe in seconds, and from there transport distances and times are short, defined by the pore size rather than the droplet size. Our coating has two levels of structure: (A) a porous scaffold and (B) an antimicrobial coating within the pore structure containing the active ingredient. Two scaffolds are studied: stainless steel and poly(methyl methacrylate) (PMMA). The active ingredient is electrolessly deposited copper. To enhance adhesion and growth of copper, a layer of polydopamine (PDA) is deposited on the scaffold prior to deposition of the copper. This porous copper coating kills 99.84% of *Pseudomonas aeruginosa* within 3 min, which is equivalent to a half-life of 27 s. In contrast, the same layer of PDA/copper on a nonporous coating kills 79.65% in the same time frame, consistent with the hypothesis that the killing rate is increased by the addition of porosity. Using the porous PMMA scaffold, the porous antimicrobial coating kills >99.99% *P. aeruginosa* in 5 min, which is equivalent to a half-life of 21 s. The higher rate of kill on the porous antimicrobial solid is appropriate for hindering the spread of infectious agents on common-use objects.

KEYWORDS: porous, polydopamine, copper, antibacterial, antimicrobial, coating, bacteria



1. INTRODUCTION

Microbial diseases kill millions of people annually and cause immense economic damage.^{1–5} One method of transmitting infectious agents (e.g., bacteria and viruses) is via solid surfaces (“fomite transmission”).^{6,7} By addition of antimicrobial coatings, we expect to reduce transmission and therefore to reduce both the incidence of morbidity and mortality and the need for antibiotic treatments. Antibiotic-resistant pathogens^{8–10} are the most troublesome because treatment options are more limited. A recent report indicated that 1.2 million people die annually due to antibiotic-resistant bacteria.¹⁰ Antibiotic resistance is sometimes called the “silent pandemic” as it causes a very high level of mortality and morbidity while receiving little attention by the media.¹¹ A Special Report^{12,13} from the US Centers for Disease Control and Prevention (CDC) recently discussed that drug-resistant infections and deaths increased during the COVID-19 pandemic. Such infections occur both in hospitals¹⁴ and in the wider community.

Multidrug-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) is a well-known bacterium that is categorized in both Healthcare-Acquired Infections and Community-Acquired

Infection and therefore is considered a “Serious Threat” by the CDC.^{15–17} Infections caused by *P. aeruginosa* are difficult to treat due to the intrinsic capability of the bacterium to adapt to new environments and acquire resistance to new antibiotics.¹⁸

P. aeruginosa bacterial cells can survive and remain infectious on solids for very long periods of time, from a few days to months.¹⁹ Also, the bacterium can be transferred to humans from contaminated surfaces.^{20,21} Therefore, *P. aeruginosa* is a good target for restricting infection through killing bacteria on common-use surfaces and is the test organism in this study. The traditional method for disinfecting surfaces is to use disinfectants such as 70% ethanol or bleach. A major problem with that approach is that contamination can reoccur soon

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after the ethanol evaporates. Another major problem is that some disinfectants, including bleach, are hazardous.²²

Antimicrobial surface coatings provide an alternative to traditional disinfectants such as bleach. A recent study showed that healthcare units that employ antimicrobial coatings have significant improvement in patient outcomes as well as reductions in environmental contamination.²³ Unlike traditional disinfectants, antimicrobial coatings have the potential to provide an ongoing kill for days, months, or even years without human intervention, although they likely still require cleaning to prevent fouling of the surface. To be effective, the coating should also provide a quick kill. The time to kill should ideally be less than the time between users of the same object, which could be minutes or even seconds. Although many existing coatings are effective,^{24–34} they would be more effective at reducing transmission if they could kill microbes more quickly. Therefore, a major research objective is to obtain both a fast kill and an ongoing kill. A typical standard for killing is 99.9%, and our objective is to achieve this in only a few minutes. For comparison, the typical standard for the US Environmental Protection Agency (EPA) is a reduction of 99.9% of microbes compared to an uncoated solid in 1 h.^{35,36}

Our hypothesis is that a porous hydrophilic coating will provide a faster kill than a flat coating of the same material. Killing requires contact between the microbe and the antimicrobial, so the transport time for the bacterium or the antimicrobial is part of the overall killing time. When a droplet lands on an impermeable solid, one component must diffuse through the droplet, which is typically on the order of 100 μm to several millimeters in size. If instead the droplet is immediately imbibed (infiltrated) into a porous coating, the relevant length scale for transport is the pore size, which is controllable and can be made smaller than the drop size. For example, it is simple to make materials with pore sizes of 100, 10, or 1 μm . Diffusion times depend on the pore geometry but typically scale faster than diffusion lengths, so a reduction in the diffusion length from 1000 to 10 μm will result in a greater than 100-fold reduction in the diffusion time. A porous antiviral coating was previously explored for a very mild antimicrobial CuO material,²⁹ but the antiviral activity was not compared to a nonporous coating.

To rationally speed the killing of bacteria, we need to examine all of the relevant time scales. The relevant time scales in this problem are the time between users of the coated item, the transport time within the droplet, the droplet imbibition time, the droplet evaporation time, and the “intrinsic” killing time for the bacterium when the active ingredient is already present at the surface of the bacteria. Our goal is to minimize the other times so that killing can approach the intrinsic killing time. The diffusion time within a droplet depends on what is diffusing: small molecules will take minutes to 1 h to diffuse 1 mm across a droplet, and a nonmotile bacterium will take even longer. This is too long for our desired total killing time. Evaporation will speed the contact between the antimicrobial and the bacterium in two ways: by reducing the drop volume and by introducing convection. But, reliance on evaporation is challenging because it depends on environmental factors. In our lab (approximately 22 $^{\circ}\text{C}$ and 32% humidity), 5 and 10 μL droplets take 44 and 73 min, respectively, to dry on the bench, which is too long. In more humid environments, we expect longer evaporation times.

Once the bacterial suspension has imbibed into a porous coating, diffusion will be fast because of the smaller distance.

However, the imbibition time also needs to be faster than our target time of a few minutes to realize an advantage from the more rapid imbibition. Imbibition times, t_i , can be estimated from the Washburn equation, which applies to a single straight pore³⁷

$$t_i = \frac{2\eta}{\gamma r_p \cos \theta} L^2 \quad (1)$$

where η is the liquid viscosity, γ is the vapor–liquid interfacial tension, r_p is the radius of the pore, θ is the contact angle of liquid on the nonporous solid, and L is the distance traveled. Imbibition times of less than 1 min are easily achievable if we use a hydrophilic solid with large pores (greater than a few micrometers). Therefore, we expect to have the droplet imbibition, diffusion, and intrinsic killing times all below the target of a few minutes.

In addition to exhibiting more rapid killing, we expect that porous coatings will have the following benefits compared to flat surfaces:³¹ (1) increased surface area for more active material, (2) protection of the active ingredient from loss due to abrasion, and (3) less microbial transfer from the surface to fingers.⁷

Here, we used a modular approach to the preparation of porous antimicrobial coatings in which we purchased or prepared a porous coating and then coated the interior pore space with an antimicrobial. We investigated two porous structures: stainless steel and poly(methyl methacrylate) (PMMA). For the antimicrobial coating we used copper on polydopamine (PDA), which is already known to kill both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*P. aeruginosa*) bacteria and to inactivate Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).²⁷ Copper usually occurs with an oxidized surface, and copper and its oxides are very broad spectrum antimicrobials and therefore useful for inhibiting transmission of a broad spectrum of microbes.^{26,38} A simple two-step procedure was followed:²⁷ first, a thin, conformal adhesion layer of PDA was deposited on the interior of the porous structure; then, copper was deposited on this layer. PDA has many advantages;^{39,40} for example, it is a naturally derived and safe material, it only requires water as its polymerization solvent, it polymerizes on virtually any solid, and it is hydrophilic, which allows penetration of microbial droplets (if the pore space is not covered by the antimicrobial). Being a thin layer, we expect PDA to cause minimal blockage of pores. Metals and metal oxides are commonly used in antimicrobial coatings,^{41–45} and here, we use copper,⁴⁶ which was deposited on the PDA by electroless deposition and created an even and conformal layer of copper. Prior work shows an outer oxidation layer on the surface of the copper, consistent with some Cu^+ .²⁷

The porous coatings had excellent antimicrobial properties: within 3 min, they reduced the number of colony-forming units (CFUs) of bacteria by 99.83% on porous antimicrobial stainless steel, which is superior to the 77.82% achieved on the nonporous antimicrobial steel and very close to our target kill rate.

2. RESULTS AND DISCUSSION

2.1. Pore Structure Is Retained after Addition of the PDA/Cu Layer. The original stainless steel (SS) scaffold porosity is heterogeneous, but even the small pores have dimensions > 10 μm , which is enough for passage of almost all

typical microbes (Figure 1A, further images in Figure S2 in the Supporting Information). The porous antimicrobial SS was

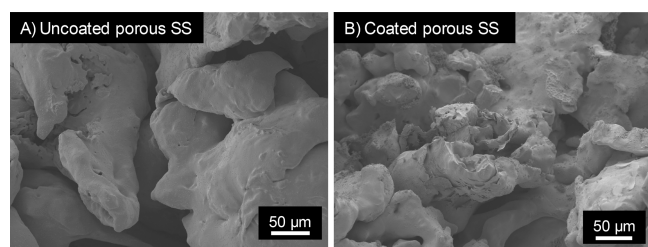


Figure 1. Cross-section SEM images of the porous stainless steel (A) without and (B) with the PDA/Cu coating.

prepared by first depositing a layer of polydopamine via solution and then copper via electroless deposition onto an SS scaffold. The presence of the copper layer on the interior porosity was confirmed by EDS, as shown in Table S1. The amount of copper on porous SS is smaller than on the flat sample as measured by EDS (see Table S1), but the surface composition as measured by XPS is similar (see Table S2). The antimicrobial layer is mainly conformal, as demonstrated in Figures 1 and S3. The porosity is thus retained after the antimicrobial coating is added. Some additional roughness can be seen on the coated structure, but the SEM images show that the effect is minor and therefore is not enough to impede passage of bacteria. Antimicrobial coatings were also prepared on flat SS surfaces to act as nonporous controls. SEM images for these flat surfaces are shown in Figure S4. The small additional roughness from the antimicrobial coating is also evident on the flat control surface.

2.2. Porous PDA/Cu Coating Allows for Very Rapid Imbibition and Drying of Droplets. The porous PDA/Cu coating is hydrophilic; on the flat PDA/Cu surface the advancing angle is $71 \pm 7^\circ$ (standard deviation) and the receding angle is $9 \pm 4^\circ$, which is suitable for imbibition of aqueous solutions. When a droplet (10 μ L) is placed on the porous material, imbibition occurs within <0.5 s, a much smaller time than our target time for killing, so imbibition will not be the limiting process (Figure 2).

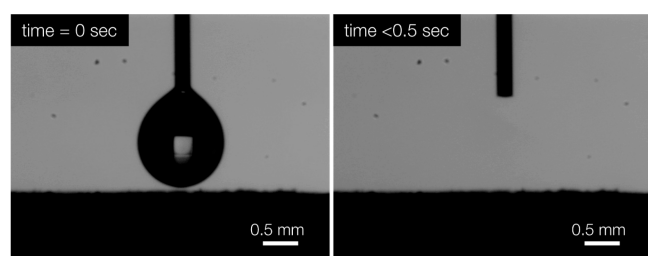


Figure 2. Photographs showing the time for a 10 μ L water droplet to imbibe into the PDA/Cu-coated porous stainless steel structure.

We also measured the drying of droplets of the porous coating. A 5 μ L droplet typically dried in about 11 min on the porous SS surface, which is much faster than the 45 min for drying on the flat coating under the same conditions. Faster drying on thin porous coatings has been noted previously,⁴⁷ and this is a significant advantage of a porous coating. A layer of water on the solid provides an additional diffusion distance for suspended bacteria in an incoming contaminated droplet, so it is better if the solid naturally evaporates water more

quickly. The “reload time” on a thin porous surface is thus shorter than that for an impermeable solid.

2.3. Porous PDA/Cu Stainless Steel Is Highly Antimicrobial. We measured the colony-forming units (CFU) of *P. aeruginosa* that were extracted from the porous PDA/Cu stainless steel as a function of time since the bacterial suspension droplet was placed on the solid, and results are shown in Figure 3. Over the very short time period of 3–5 min, the CFU level dropped by about 3 orders of magnitude, and therefore, the porous PDA/Cu is a very effective antimicrobial material.

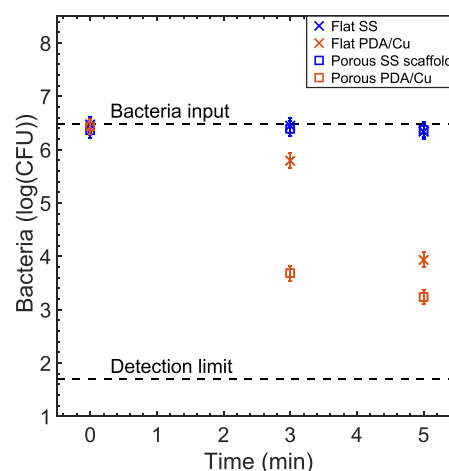


Figure 3. Time course of *P. aeruginosa* on flat PDA/Cu and porous PDA/Cu coatings. Compared to the control SS, the porous PDA/Cu coating causes a reduction of 99.83% of *P. aeruginosa* within only 3 min, while the same coating in a flat configuration causes a 77.82% reduction (see eq 3 in the Experimental Section for the equation for reduction). The bacteria input on the coatings and the detection limit for the bacterium are shown with dotted lines. Each symbol represents the average of two independent samples. A 3-way ANOVA gave the following *p* values: porosity (porous vs flat), *p* = 0.008; surface type (uncoated vs coated sample), *p* < 10^{-6} ; time, *p* < 10^{-4} . The 2-way interactions are as follows: *p*_{surface type and time} < 10^{-4} , *p*_{surface type and porosity} = 0.0135, *p*_{time and porosity} = 0.0436. The 3-way interaction *p* value between all three factors is 0.038, which indicates that over time, the porosity makes a difference if the antimicrobial coating is present. The points are the average of three measurements and the error bars are the least significant difference from the ANOVA.

The effectiveness of the surface treatment is usually considered by comparison of the surface treatment to some standard. Two different standards are frequently considered: (1) the input level of bacteria or (2) a control surface at the same time. Comparison to the input level gives us the metric of “killing” defined in eq 4 of the Experimental Section. Comparison to a control surface is important because bacteria may slowly die on their own without an antimicrobial, and we use the term “reduction” as defined in eq 6 in the Experimental Section.

The porous PDA/Cu kills 99.84% of *P. aeruginosa* within 3 min. The effective half-life is 27 s (with 95% confidence interval boundaries: 17–73 s), calculated by assuming exponential decay for the first 5 min. We now compare this result to various controls. The flat PDA/Cu surface kills 79.65% of *P. aeruginosa* within 3 min, so the number of surviving bacteria at this time point was reduced by a factor of 127 by introducing the porosity. Our statistical evidence is a 3-

way ANOVA (factors: time, porosity, and antimicrobial) where we assess whether the addition of porosity gives an increased kill over time with a 3-factor interaction term. The 3-factor interaction has $p = 0.038$, so we consider this interaction significant, and this is consistent with the hypothesis that a porous antimicrobial coating kills faster than a flat surface. We attribute the additional killing to the increased interfacial area of the antimicrobial component (i.e., copper) and the shorter diffusion transport distance.

Antimicrobial coatings are usually assessed by the percent reduction compared to a control (uncoated surface) as shown in eq 6, and such a comparison is required by the EPA.^{35,36} In this case, we use the flat SS as the comparison, and Figure 3 indicates that flat SS kills few bacteria. The porous PDA/Cu coating causes a reduction of 99.83% of *P. aeruginosa* within only 3 min, while the same coating in a flat configuration causes a 77.82% reduction. The 95% confidence interval for the log reduction compared to the flat SS is 2.76 in 3 min. To be registered by the EPA, a coating needs a 3-log reduction in 1 to 2 h, whereas the porous material achieves a similar level in only a few minutes.

From Figure 3, it is clear that the number of viable bacteria that survive exposure to the porous material depends strongly on the presence of the antimicrobial. For example, after 5 min only 0.05% of the original inoculum is viable on the porous coating containing the antimicrobial, whereas 80.5% was viable on the porous material without the antimicrobial. Comparing these two results we know that the low CFUs observed for the porous antimicrobial coatings are not simply due to trapping of the bacteria on the solid.

2.4. Our Assay Is Effective at Extracting Bacteria from the Porous Coating. A question naturally arises as to whether bacteria can be extracted from the porosity. If bacteria were to be trapped then they would not show up in our colony-forming unit (CFU) assay and there may be ambiguity as to whether our porous antimicrobial layer traps or kills bacteria. We do note that if bacteria were trapped then such trapped bacteria would not be available for transfer to humans, and being trapped on an antimicrobial surface is not an ideal location for a bacterium to replicate.

We have deliberately selected large pores to limit trapping of microbes, but we investigated the extent of trapping. It is difficult to test trapping separately from killing on an antimicrobial surface, so we tested trapping on the same microstructure but with two different chemistries, stainless steel and PDA, both of which have no antimicrobial coating. Clearly, both the chemistry and the time in contact could in principle affect the extraction. A suspension of bacteria was placed on the porous coating, and then, the bacteria were extracted by the same procedures later used for killing (sonication and vortexing). Five minutes inside the coating was used as the test period because this was our approximate target time for killing by the active material. Figure 4 compares the measured CFU after extraction to the CFU obtained from the test droplet: almost all bacteria inside the pores of the porous scaffolds were successfully extracted. Comparing the PDA and SS chemistries, there is no effect of the surface chemistry on trapping ($p = 0.192$). The lack of killing also shows that neither the bare PDA nor SS is a good antimicrobial surface. The ability to extract bacteria from planar surfaces was also high (Figure S5). We also tested the trapping effect on a variety of drop sizes. Bacteria were successfully extracted from the porous scaffold when 10, 30, and 50 μL volumes of

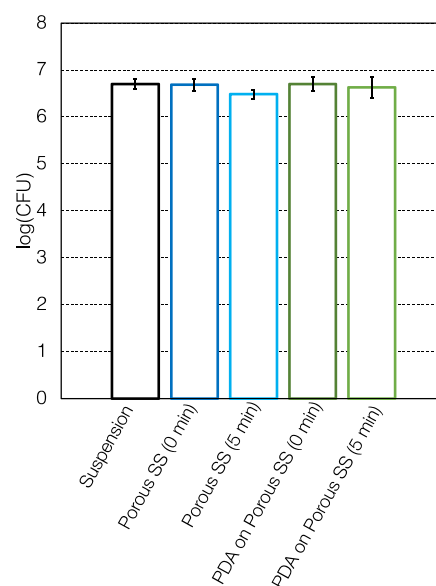


Figure 4. CFUs of *P. aeruginosa* extracted from solids compared to the CFUs input on the solid. A very high percentage of bacteria is extracted from the porous coating. The detection limit is 50 CFU. There is a small but not significant ($p = 0.071$) decrease in the CFU for porous stainless steel compared to the input, but this also occurs for flat stainless steel (see Figures 3 and S5). After the porous structure was coated with PDA, the bacteria were still extracted ($p = 0.44$ comparing input and extracted).

suspension were used (see Figure S6 in the Supporting Information).

2.5. Porous Antimicrobial Polymer Coating. Polymer coatings can easily be added to surfaces, the most obvious example of which is latex paint, in which polymer particles are applied to a solid and then chain migration ripens the particles into a continuous film. Here, we make a $\sim 110\ \mu\text{m}$ thick porous antimicrobial coating by a similar method, except that we choose a polymer with a glass transition temperature, T_g , above room temperature. With this feature we can control a period of elevated temperature such that ripening is halted when the polymer film is continuous, but the porosity is still present. We chose PMMA particles because PMMA has a relatively low T_g ⁴⁸ and heated the particles at 125 $^{\circ}\text{C}$ for 2 h. We chose a 1–40 μm particle size range, so that large pores could form for passage of bacteria and rapid imbibition. PMMA also has high compressive and tensile strengths,⁴⁹ making it an attractive material for a coating. Figures 5 and S7 (in the Supporting Information) show SEM images of the fabricated porous

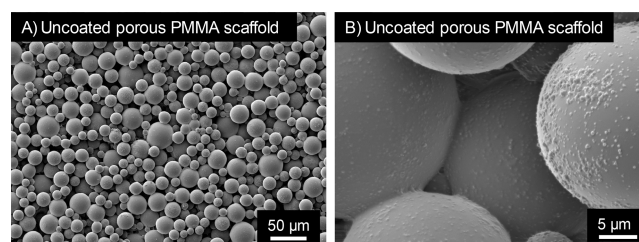


Figure 5. SEM images of a porous PMMA scaffold fabricated by partial sintering of PMMA particles through 2 h of heat treatment at 125 $^{\circ}\text{C}$ (above T_g). (A) Image showing the retained porosity. (B) Image showing the formation of polymer “necks” or frozen mensci. Further images are in Figure S7.

PMMA scaffold. High-magnification images (Figure 5B) show the formation of necks after heat treatment, which indicate that polymer migration has occurred to form connections between the particles, and therefore a continuous coating, but the porosity has not been lost.

Water does not penetrate uncoated porous PMMA—the advancing and receding angles of a flat surface are $111 \pm 4^\circ$ (std dev) and $16 \pm 2^\circ$, respectively. After deposition of a PDA layer, the coating becomes hydrophilic and imbibes (infiltrates) water droplets in seconds. CFU measurements (Figure S8 in the Supporting Information) show that neither flat nor porous PMMA scaffolds are good antimicrobial coatings.

The antimicrobial PDA/Cu layer was prepared within the pores of the porous PMMA. SEM images confirmed that porosity was retained (see Figure 6); EDS confirmed the

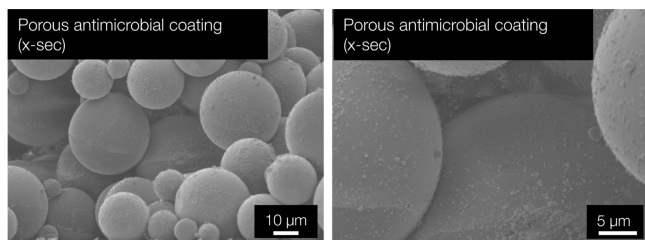


Figure 6. SEM images of the porous antimicrobial coating (scaffold = PMMA). The antimicrobial layer (PDA/Cu) was successfully prepared inside the pores of the porous PMMA scaffold.

presence of the copper in the coating (see Table S3), and imbibition was achieved in <0.5 s (see Figure S9 in the Supporting Information), which together make this coating suitable for examining killing in a porous antimicrobial coating. A control experiment showed that PDA is not the active ingredient (Figure S10). Additionally, a $5 \mu\text{L}$ water droplet dries in about 5 min on PDA/Cu-coated porous PMMA, whereas the same volume takes about 45 min to dry on a flat surface.

Figure 7 shows the time course of *P. aeruginosa* CFU on the porous PDA/Cu, which was made using the porous PMMA scaffold. The coating is highly antimicrobial; it kills 99.27% and $>99.99\%$ of *P. aeruginosa* in 3 and 5 min, respectively. To compare the coating to the uncoated control, the coating causes a reduction of 99.02% and $>99.99\%$ of the bacterium in 3 and 5 min ($p < 0.01$), respectively. The log reduction in 5 min is 4.5 ± 0.6 (95% confidence interval). The effective half-life is 21 s (with 95% confidence interval boundaries: 18–26 s; calculated over 5 min). Our hypothesis was that adding porosity would improve killing speed, and when we compare the porous coating to the flat coating, we see the killing is increased from 79.65% to 99.02% in 3 min and from 99.71% to $>99.99\%$ in 5 min, which is consistent with the hypothesis ($p < 0.001$ for coating-time interaction in a 2-way ANOVA). Again, we attribute the fast killing to the short diffusion distances and the high surface area of the antimicrobial layer.

2.6. Abrasion Resistance. In principle, a huge advantage of a porous antimicrobial coating should be improved resistance to abrasion. This abrasion resistance can arise from two avenues. First, one can choose the scaffold independently from the antimicrobial, so that an abrasion-resistant scaffold can protect the antimicrobial material. Second, abrasion affects only the outer layer, so even when the outer layer is abraded, the inner layer is retained. To test

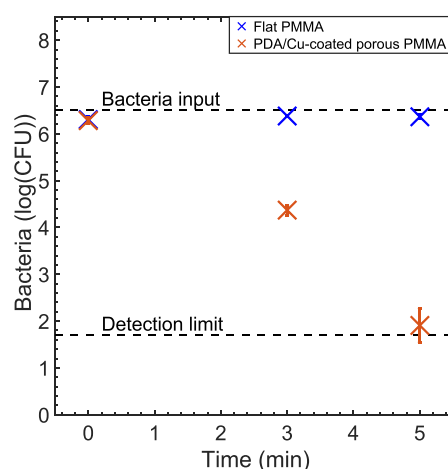


Figure 7. Time course of bacteria on uncoated flat PMMA (control) and PDA/Cu-coated porous PMMA. The porous PDA/Cu coating is highly effective. The porous PDA/Cu causes a reduction of $>99.99\%$ of *P. aeruginosa* in 5 min compared to the uncoated sample ($p = 0.009$). Each symbol represents the average of two separate samples, and the error bars are standard deviation. The bacteria input on the coatings and the detection limit for the bacterium are shown with dotted lines. To determine the effect of porosity, we compared the PDA/Cu-coated porous material to the flat PDA/Cu material on SS, assuming that the underlying support was not important. For a 2-way ANOVA, factors of sample ($p < 0.001$), time ($p < 0.001$), and sample–time ($p < 0.001$) were significant, providing additional support for the hypothesis that porosity speeds the killing of *P. aeruginosa*.

this idea, we abraded the SS antimicrobial coating with a sponge using a modified EPA test,³⁵ and the results are shown in Figure 8. After abrasion, the antimicrobial properties were

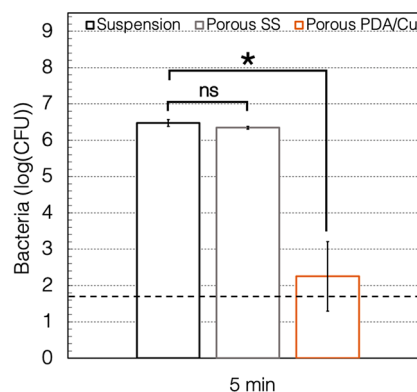


Figure 8. Bacteria tests with abraded porous PDA/Cu coating. The abraded coatings kill 99.9% of bacteria within 5 min. The detection limit (i.e., all bacteria were dead) is 50 CFU (asterisk (*) represents $p < 0.05$; ns represents $p > 0.05$).

retained and 99.9% of the bacteria were killed. This is not surprising as one would not expect a sponge to abrade the inner layer.

2.7. Other Characteristics of the PMMA Coating. We have used convenient conditions and low-toxicity materials. Creation of the PMMA scaffold occurs at low temperature and a short duration (125°C for 2 h). In principle, a lower T_g material could be used, providing it was above room temperature. Dopamine polymerization is completed in water, and therefore, no harmful organic solvents are required.

Throughout the process, there is no specialized equipment. In addition, PDA has a high median lethal dose (LD_{50}).⁵⁰ Copper and PMMA are inexpensive materials, and only a very thin layer of PDA is used. One can imagine other antimicrobial materials being added to a scaffold, such as Cu_2O or Ag_2O particles, providing that the particles do not completely block the porosity. Thus, the porous scaffold plus active agent is modular where each one can be optimized separately.

3. CONCLUSION

For maximum effectiveness, an antimicrobial coating should kill microbes between users. Common-use objects, such as doorknobs, are sometimes touched by different individuals within a period of minutes or even less, so the kill needs to be swift. We hypothesized that the rate of kill of an antimicrobial coating was transport limited due to millimeter transport distances within droplets and that a faster kill could be obtained with shorter transport distances. To achieve shorter transport distances, we devised the idea of porous hydrophilic antimicrobial coatings, where a droplet contaminated with microbes that lands on the solid would quickly penetrate into the porosity and then the transport distance would be the pore size. We fabricated two examples of porous antimicrobial surfaces using PDA/Cu as the active material and found very rapid killing. For a porous SS scaffold, 99.84% of *P. aeruginosa* was killed in 3 min. The effective half-life of *P. aeruginosa* in the liquid within the porosity was 27 s. For porous PMMA, >99.99 of *P. aeruginosa* was killed within 5 min and the effective half-life of the bacterium was 21 s. We demonstrated that the antimicrobial material was more effective on a porous scaffold than on a flat impermeable solid ($p = 0.038$ for the 3-way interaction on stainless steel and $p < 0.001$ for the 2-way interaction on PMMA), consistent with our hypothesis of transport-limited inactivation on the flat antimicrobial surface. The larger surface area of active material per volume of liquid within the porous material may have also contributed to the kill, and this is an additional advantage of the porous geometry.

A further advantage is that the inner active layers can be protected from abrasion by the outer layers. We tested the latter idea by doing a standard abrasion test on the porous coating and found that the coating was still active after abrasion. The use of a modular scaffold plus active ingredient system allows an independent choice of the porous scaffold from the antimicrobial material. We demonstrated this with the use of two scaffolds: stainless steel and PMMA. Both scaffolds had similar, excellent, antimicrobial activity when used with the same antimicrobial coating layer.

A critical aspect of the porous layer is that the microbial droplet should absorb into the solid quickly. This is simple to achieve providing that the porosity is large (greater than a few micrometers) and the pore space is hydrophilic. It is not necessary for the structural component of the scaffold to be hydrophilic providing that the antimicrobial coating is sufficiently hydrophilic. This was demonstrated with PDA/Cu-coated porous PMMA.

Specific advantages of our choice of materials are that we used common, inexpensive, and relatively nontoxic materials (Cu, PDA, PMMA, stainless steel) and the procedures were simple and required no specialized equipment.

4. EXPERIMENTAL SECTION

4.1. Materials. Seventy percent ethanol and sodium hydroxide beads (ACS grade) were purchased from VWR. Dopamine hydro-

chloride (99%) and dimethylamineborane (DMAB, 98%) were obtained from Fisher Scientific. Flat stainless steel (SS), porous SS (Catalog No. = 9446T32, diameter = 12.7 mm, thickness = 1.6 mm), and flat acrylic (PMMA; Catalog No. = 4615T61) were obtained from McMaster-Carr. The following were purchased from Sigma-Aldrich: copper(II) chloride (97%), boric acid (ACS grade), tris(hydroxymethyl)aminomethane (Tris, ACS reagent), ethylenediaminetetraacetic acid (EDTA, >99%), and sodium dodecyl sulfate (SDS, >99%). PMMA particles (PMMA microspheres, 1–40 μ m) were purchased from Cosphere. Water was purified by a Milli-Q Reference water purification system. Flat SS or porous stainless steel was cleaned using soapy water, DI water, and 70% ethanol and finally rinsing with DI water. After preparation of the antimicrobial coating, the coated porous SS was fixed on a flat SS using double-sided tape. Bacterial cell suspensions were diluted in either phosphate-buffered saline (PBS) or DE broth (BD, Sparks, MD). Tryptic soy agar (TSA) and tryptic soy broth (TSB) were obtained from BD (Sparks, MD).

4.2. Fabrication of Porous PMMA Scaffold Test Samples. 80 μ L of PMMA particle suspension (15% w/w in water with 8 mM SDS) was drop cast on flat acrylic (size = 12 $\times$ 12 mm). The samples were left at room temperature to dry. Then, the samples were placed in a furnace at room temperature, and the temperature was increased to 125 $^{\circ}$ C at a rate of 2 $^{\circ}$ C/min. After 2 h at 125 $^{\circ}$ C, the furnace was turned off and the samples were cooled to room temperature. The samples were washed three times with DI water (6 min each) to remove residual SDS. The thickness of the porous PMMA scaffold was about 110 μ m.

4.3. Deposition of Antimicrobial PDA/Cu Surface Coating.

The PDA and Cu deposition both use dissolved molecular reagents, so the deposition is not affected by being confined in pores that are greater than one micrometer in size. However, it is necessary for the water solvent to penetrate the porosity, and this requires that the interior surfaces are hydrophilic. Steel is sufficiently hydrophilic to allow imbibition, but PMMA is hydrophobic, so the interior pores of the PMMA samples were rendered hydrophilic by plasma treatment with oxygen for 3 min at 100 W. Preparation of the antimicrobial coating had two steps:²⁷ deposition of the adhesive layer (polydopamine; PDA) followed by deposition of copper via electroless deposition. PDA^{39,51} was deposited as follows: on a stirring hot plate, tris buffer (10 mM) was heated to 60 $^{\circ}$ C, after which dopamine hydrochloride was added (final concentration = 5 g/L). The samples were then submerged in this aqueous liquid for 4 h while stirring. After deposition, the container was subsequently removed from the hot plate and allowed to cool to room temperature. The samples were washed with DI water and dried with N_2 gas.

For electroless deposition of copper on the PDA,^{27,39,52–55} an aqueous solution of EDTA (50 mM), $CuCl_2$ (50 mM), and boric acid (100 mM) (pH = 7 using NaOH) was stirred at 38 $^{\circ}$ C while DMAB was added (final concentration = 100 mM). The PDA-coated samples were submerged in this solution and kept for 160 min after the solution color changed to dark green. The porous solids were hydrophilic, so the coating solution was rapidly imbibed into the porosity and the copper deposition occurred on the interior and exterior of the surfaces. The samples were removed, washed with DI water, and dried with N_2 gas.

4.4. Characterization. Characterization of the morphology of the coatings was achieved by SEM images using a JEOL IT500 SEM. Samples for SEM imaging (only) were treated with a 5 nm layer of Pt/Pd. Advancing and receding water contact angles were measured with a First Ten Angstroms FTA125. Void volume percents of the porous SS and porous PMMA scaffolds were $33 \pm 3\%$ (standard deviation) and $53 \pm 1\%$, respectively.

4.5. Antibacterial Assay. **4.5.1. Choice of Microbial Strain.** Following the guidance of the US EPA,^{35,36} *P. aeruginosa* strain DSM-9644 was chosen for the experiments.

4.5.2. Growth of Microbial Strains. *P. aeruginosa* strain (DSM-9644) was grown in TSB (5 mL) to midexponential phase at 37 $^{\circ}$ C with aeration (at 60 rpm). The purity and identity of the cells were verified by streaking bacteria from these liquid cultures on TSA plates,

incubating at 37 °C for 48 h, and then examining these colonies for species-specific traits (such as surface texture and pigmentation).

4.5.3. Preparation of Microbial Strains for Testing. Bacterial cells from the cultures were collected by centrifugation for 20 min at 5000g. The supernatant was then discarded, and the bacterial cells were resuspended in sterile PBS (5 mL) using vortexing for 60 s. The said suspensions were centrifuged for 20 min at 5000g, the supernatant was discarded, and the washed cells were resuspended in sterile PBS (5 mL) using vortexing for 60 s.

4.5.4. Measurement of Viable Cell Number. The viable number of cells in the PBS suspensions of the bacterium was measured as colony-forming units per milliliter (CFU)/mL of suspension as follows: A 10-fold dilution series was prepared for each suspension in either PBS or DE broth, 0.1 mL of each dilution was spread on TSA plates (in triplicate), and colonies were counted after 48 h of incubation at 37 °C for the dilution that produced a convenient number to resolve (typically 20–200 colonies). If no colonies were present for the lowest dilution then to enable a $\log_{10}$ transformation of all data, we designated the zero as one. This is our detection limit for measurement on a particular plate.

4.5.5. Measurement of Surface Killing. For each named bacterial strain, a droplet of bacterial cells in PBS was placed on individual coated or uncoated sample. The droplet size was 10 μ L for flat or porous SS samples and 5 μ L for flat or porous PMMA samples. Immediately, after specific time points, each sample was placed in a separate sterile 50 mL centrifuge tube containing 5 mL of DE broth (or PBS where noted), vortexed for 10 s at the highest setting, and sonicated for 1 min (Branson model 12 Ultrasonic Cleaner, Shelton, CT) to detach cells from the solid. DE was chosen because it is known to neutralize metals.⁵⁶ The CFU/mL of the resulting bacterial suspension was measured by removing a 0.1 mL sample of the suspension without coming in contact with the coated or uncoated sample, preparing a dilution series, plating on TSA plates, and incubating at 37 °C for 48 h at 37 °C. This process was repeated at each specific time point, and independent samples were used for each condition, i.e., each coated or uncoated sample at each time point. Note that the test droplet contacts only one side of the samples. The detection limit and resolution of plate counting is 1 colony, but because the original bacterial sample droplet of 10 or 5 μ L was diluted to 5 mL in the centrifuge tube and from this only 0.1 mL was sampled, the detection limit of bacteria from the original test droplet was 50 CFU.

4.6. Abrasion Tests. The porous coatings were abraded by an abrasion tester (model Gardco D10 V) using a mounted sponge. This was based on the EPA protocol,³⁵ which is utilized to evaluate antimicrobial coatings for EPA approval. This protocol has abrasion with a sponge wetted with an antimicrobial agent (70% ethanol). The tester machine moves back and forth a sponge that is in contact with the samples with an amplitude of $\sim$ 26 cm and a period of approximately 5 s. The sponge was loaded with a load of 0.45 kg during the tests, and it was wetted with 20 mL of 70% ethanol before the abrasion cycles. An abrasion cycle was done as follows: the sponge was moved across the sample 8 times; subsequently, 70% ethanol was sprayed on the abrasion platform and the samples with a subsequent waiting period (30 min). A total of 10 cycles (equal to 80 passes) was performed on the samples. A single sponge was used for the first 5 abrasion cycles, and it was replaced with a separate sponge for the second 5 abrasion cycles.

4.7. Statistical Analysis. We carried out bacterial assays in a randomized manner (with randomized factors), and our statistical power was at least 80% for each factor at the significance level of 0.05. All statistics were calculated from log CFU, and Student's *t* tests were two-tailed and heteroscedastic. Error bars are standard deviation unless otherwise noted.

4.8. Calculation of Microbial Reduction. The word “killing” in the Results and Discussion is used for simplicity; we recognize that the CFU counting assay actually measures those cells that can reproduce to form a colony, but we refer to any cell that does not produce a colony as having been killed. Killing is defined as

$$\log \text{ killing} = \text{mean} \left[\log_{10} \left(\frac{\text{input titer}}{\text{units}} \right) \right] - \text{mean} \left[\log_{10} \left(\frac{\text{sample titer}}{\text{units}} \right) \right] \quad (2)$$

$$\% \text{ killing} = [1 - 10^{-\log \text{ killing}}] \times 100 \quad (3)$$

$$\% \text{ survival} = 1 - \% \text{ killing} \quad (4)$$

The term “reduction” compares CFU measurement for two surfaces, an uncoated and a coated surface, at the same time

$$\log \text{ reduction} = \text{mean} \left[\log_{10} \left(\frac{\text{uncoated surface titer}}{\text{units}} \right) \right] - \text{mean} \left[\log_{10} \left(\frac{\text{sample titer}}{\text{units}} \right) \right] \quad (5)$$

$$\% \text{ reduction} = [1 - 10^{-\log \text{ Reduction}}] \times 100 \quad (6)$$

The half-life was calculated from a linear fit of the slope of all log (CFU)–time data for a particular condition. The half-life is

$$t_{1/2} = -\frac{0.30}{\text{slope}} \quad (7)$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsami.2c22240>.

Schematic of abrasion tests on the coatings; SEM images of porous SS scaffold; elemental characterization (EDS) of x-section of porous PDA/Cu; plan-view and cross-section SEM images of coated porous SS; plan-view SEM images of PDA/Cu-coated flat SS surfaces; bacterial CFUs extracted from flat and porous scaffolds; extraction of *P. aeruginosa* from porous SS as a function of droplet volume; SEM images of porous PMMA scaffold; time course of bacteria CFU on flat and porous PMMA scaffolds; photographs showing the time for a water droplet to imbibe into the PDA/Cu-coated porous PMMA; time course of *P. aeruginosa* CFU on PDA-coated porous PMMA (PDF)

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Notes

The authors declare the following competing financial interest(s): W.A.D. declares part ownership in a startup company that intends to produce surface coatings. Other authors declare no conflict of interest.

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