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Human upper airway epithelium produces nitric oxide in response to *Staphylococcus epidermidis*

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Abstract

Background—Nitric oxide (NO) is produced by sinonasal epithelial cells as part of the innate immune response against bacteria. We previously described bitter-taste-receptor-dependent and -independent NO responses to product(s) secreted by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. We hypothesized that sinonasal epithelium would be able to detect the gram-positive, coagulase-negative bacteria *Staphylococcus epidermidis* and mount a similar NO response.

Methods—Sinonasal air-liquid interface cultures were treated with conditioned medium (CM) from lab strains and clinical isolates of coagulase-negative staphylococci and *S aureus*. NO production was quantified by fluorescence imaging. Bitter taste receptor signaling inhibitors were utilized to characterize the pathway responsible for NO production in response to *S epidermidis* CM.

Results—*S epidermidis* CM contains a low-molecular-weight, heat, and protease-stable product that induces an NO synthase (NOS)-mediated NO production that is less robust than the response triggered by *S aureus* CM. The *S epidermidis* CM-stimulated NO response is not inhibited by antagonists of phospholipase C isoform β -2 nor the transient receptor potential melastatin isoform 5 ion channel, both critical to bitter taste signaling.

Conclusion—This study identifies an NO-mediated innate defense response in sinonasal epithelium elicited by *S epidermidis* product(s). The active bacterial product is likely a small, nonpeptide molecule that stimulates a pathway independent of bitter taste receptors. Although the NO response to *S epidermidis* is less vigorous compared with *S aureus*, the product(s) share similar characteristics. Together, the responses to staphylococci species may help explain the pathophysiology of upper respiratory infections.

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Keywords

chronic rhinosinusitis; epithelial; infection; innate immunity; nitric oxide; *S epidermidis*

Epithelial cells that line the upper airway constantly defend against inhaled pathogens and serve integral roles in both innate and adaptive immunity.^{1,2} The epithelium's role in innate immune defense includes mucociliary clearance (MCC) through beating cilia and secretion of bactericidal products.^{3,4} Nitric oxide (NO) is a molecule produced by the sinonasal epithelium that is able to prevent infection by both direct and indirect mechanisms. NO activates intracellular signaling pathways to increase ciliary beat frequency,^{5,6} thereby increasing mucociliary clearance, and it also diffuses through the airway mucus to directly damage bacterial membranes, enzymes, and DNA, and thus has direct bactericidal effects. 7–10

Previous work from our lab demonstrated that human upper airway epithelial cells are able to detect secreted products from 2 important bacteria believed to contribute to chronic rhinosinusitis, gram-negative *Pseudomonas aerigonsa*¹¹ and gram-positive *Staphylococcus aureus*.¹² Secreted product(s) from both of these pathogens trigger an NO response; however, the response to gram-negative acyl homoserine lactone (AHL) is mediated by the bitter taste receptor T2R38,¹¹ and the response to *S aureus* product(s) is independent of bitter taste receptor signaling.¹² We hypothesized that other gram-positives and -negatives are able to activate NO production in the upper airway, and in this study we sought to determine whether coagulase-negative staphylococci, specifically *Staphylococcus epidermidis*, also elicit a NO response from human sinonasal epithelium and, if so, to characterize the *S epidermidis* product(s) eliciting the response and determine whether the epithelial signaling was mediated by the canonical bitter taste receptor pathway.

Staphylococci species such as *S aureus* and *S epidermidis* are common colonizers of the nasopharynx and sinuses in healthy individuals¹³ and those with chronic rhinosinusitis.^{14,15} Therefore, it is logical that the innate immune system of the upper airway would have mechanisms in place to keep these species under control and prevent disease. Similarly, it is likely that altered epithelial inflammatory responses—either too robust or insufficient—may predispose some individuals to inflammation, infection, and/or chronic rhinosinusitis. By better understanding the interaction between *S epidermidis* and the host innate immune system, the complex interplay between human respiratory mucosal surfaces and the commensal bacteria may be further defined.

Materials and methods

Sinonasal air-liquid interface cultures

Cultures were prepared as described in previous studies.^{11,16} Surgical specimens of sinonasal mucosa were acquired from patients undergoing functional endoscopic sinus surgery (FESS) at the Department of Otorhinolaryngology at the University of Pennsylvania and the Philadelphia Veterans Affairs Medical Center. The institutional review boards at both centers provided full study approval and informed consent was obtained from all

patients preoperatively. Patients were excluded from the study if they had a history of systemic diseases such as sarcoidosis, granulomatosis with polyangiitis, cystic fibrosis, and immunodeficiency syndromes, or if they had been prescribed oral corticosteroids, antibiotics, or anti-biologics (eg, omalizumab) within 1 month of surgery. Air-liquid interface (ALI) cultures were prepared by enzymatically dissociating the sinonasal tissue epithelial cells and growing them to confluence in tissue culture flasks (75 cm²) using bronchial epithelial basal medium (BEBM; Clonetics, Cambrex, East Hanover, NJ) and proliferation medium consisting of Dulbecco's modified Eagle medium (DMEM)/Ham's F11 media containing 100 U/mL penicillin and 100 µg/mL streptomycin for 7 days. The epithelial cells were treated with trypsin and seeded on porous polyester membranes (67 × 10⁴ cells per membrane) in cell culture inserts (Transwell-clear, 11-mm diameter, 0.4-µm pores; Corning, Acton, MA) coated with 100 µL of coating solution (bovine serum albumin [BSA; 0.1 mg/mL; Sigma-Aldrich, St Louis, MO] and fibronectin [10 µg/mL; BD Biosciences, San Jose, CA] in LHC basal medium [Invitrogen, Grand Island, NY]) and bovine type I collagen (30 µg/mL; BD Biosciences). The cell cultures were then placed in a tissue culture laminar flow hood for 11 hours. The culture media was removed from the apical side after 5 days and the epithelium was driven to differentiate using medium consisting of 1:1 BEBM (Clonetics; Cambrex) and DMEM (Invitrogen) with the Clonetics complements for human epidermal growth factor (hEGF; 0.5 ng/mL), bovine pituitary extract (BPE; 0.13 mg/mL), insulin (5 g/mL), hydrocortisone (0.5 g/mL), epinephrine (5 g/mL), triiodothyronine (6.5 g/mL), and transferrin (0.5 g/mL), supplemented with 100 U/mL penicillin, 100 g/mL streptomycin, 0.1 nmol/L retinoic acid (Sigma-Aldrich), and 10% fetal bovine serum (FBS; Sigma-Aldrich) in the basolateral side.

Bacteria conditioned medium

S epidermidis strain ATCC 14990 and *S aureus* strain M2 were used for preparation of *S epidermidis* and *S aureus* conditioned medium (CM), respectively. Clinical isolates of coagulase-negative staphylococci and *S aureus* were obtained from human nasal cultures for preparation of coagulase-negative staphylococci clinical isolate CM and *S aureus* clinical isolate CM. The strains and clinical isolates were each grown separately for 14 hours at 37°C with shaking in lysogeny broth (LB) medium. The 14-hour cultures were then diluted to 0.1 optical density (OD) (log phase), and grown for an additional 12 hours. The cultures were adjusted to an OD of 0.5 with LB, then centrifuged (2000g for 10 minutes at room temperature) and filtered using a 0.2-µm filter to produce the CM. The CM was plated onto LB agar plates and incubated overnight at 37°C to assure that no viable bacteria were present in the CM.

Dialysis of the *S epidermidis* CM was performed using a 3.5 kDa cutoff dialysis membrane (Spectra/Por; Spectrum Medical Industries, Inc., Laguna Hills, CA) for 5 hours at 4°C against a 1000× excess of LB that was changed at 2.5 hours. Boiled *S epidermidis* CM was prepared by heating the *S epidermidis* CM at 100°C for 1 hour followed by immediate transfer to an ice bath. Trypsinized *S epidermidis* CM was prepared using 250-µg/mL trypsin (Life Technologies) at 37°C for 1 hour followed by 5 mmol/L of 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, an irreversible serine protease inhibitor (Fisher Scientific, Waltham, MA) at 37°C for 2 hours. All bacteria CM was diluted in Dulbecco's

phosphate-buffered saline (DPBS) to a final concentration of 25%, as the equivalent concentration of LB medium was the highest concentration that did not have deleterious effects on the ALIs.

Live-cell imaging of NO production in human ALI cultures

The fluorescent reactive nitrogen species indicator 4-amino-5-methylamino-2',7'-difluorofluorescein (DAF-FM; Invitrogen/Life-Technologies) was used to quantify cellular NO production and imaging was performed as previously described.¹¹ Briefly, cells were loaded on the apical side with DAF-FM-diacetate by incubating with DPBS containing 10 $\mu\text{mol/L}$ DAF-FM-diacetate and 5 $\mu\text{mol/L}$ of the cell-permeant NO scavenger carboxy-PTIO (cPTIO; Cayman Chemical, Ann Arbor, MI). After 90 minutes, the cultures were washed 5 times with PBS to remove any unloaded DAF-FM and cPTIO and incubated for an additional 15 minutes with 30 μL of PBS on the apical side. An IX-81 microscope ($\times 10$, 0.3 NA UPlanFLN objective; Olympus) and the 488-nm argon laser line of a laser scanning confocal system (FluoView FV1000; Olympus) was utilized for imaging. After establishing a baseline fluorescence, 30 μL of the treatment was added to the apical side of the cultures, and DAF-FM fluorescence images were acquired every 5 seconds.

DAF-FM experiments were performed using modified 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES)-buffered Hank's balanced salt solution (HBSS) containing $1\times$ minimal essential medium (MEM) amino acids on the basolateral side of the cultures and DPBS (containing 1.8 mmol/L Ca^{2+}) on the apical side. Stock solutions of DAF-FM and cPTIO were prepared at 1000 $\times$ in dimethylsulfoxide (DMSO) and working solutions were made fresh in DPBS for each experiment. The phospholipase C (PLC) β -2 inhibitor, U73122, and its inactive analog, U73343 (Cayman Chemical), were used at 5 $\mu\text{mol/L}$. The transient receptor potential melastatin isoform 5 (TRPM5) blocker triphenylphosphine oxide (TPPO; Sigma-Aldrich) was used at 100 $\mu\text{mol/L}$. Working solutions of these inhibitors or inactive analogs were prepared fresh daily in PBS from 1000 $\times$ DMSO stock solutions. During the experiments, the inhibitor solutions were added to the apical side of the cultures for 15 minutes prior to DAF imaging and were contained in the *S epidermidis* CM treatments. L- N^{G} -nitroarginine methylester (L-NAME; Cayman Chemical), a nitric oxide synthase (NOS) inhibitor, was used at 100 $\mu\text{mol/L}$ and was prepared from a 1000 $\times$ DMSO stock solution on the day of the experiment. L-NAME was applied to both the apical and basolateral sides 15 minutes prior to DAF imaging and was contained in the *S epidermidis* CM.

Data analysis and statistics

FluoView software (Olympus, Tokyo, Japan) was used to analyze DAF-FM data, and GraphPad Prism (Graph-Pad Software, Inc., La Jolla, CA) was used for statistical analysis, with $p < 0.05$ considered statistically significant. The unpaired 2-tailed t tests were used for single comparisons and 1-way analysis of variance (ANOVA) with Bonferroni's posttest was used for multiple comparisons. All data are reported as mean $\pm$ standard deviation (SD).

Results

S epidermidis* CM elicits an epithelial NO response that is less robust than *S aureus

Our previous work showed NO production by upper airway epithelial cells in response to secreted products by gram-negative *P aeruginosa*¹¹ and gram-positive *S aureus*.¹² We first sought to determine whether *S epidermidis*, a gram-positive bacterium, also triggered a NO response in sinonasal epithelium. The fluorescent probe DAF-FM, which reacts with NO-derived reactive nitrogen species to form a fluorescent benzotriazole, was used to measure the cellular production of NO after treatment with *S epidermidis* CM. We determined that *S epidermidis* CM stimulation did in fact result in a rapid production of NO-derived reactive nitrogen species (DAF-FM fluorescence increase) over the course of 2 minutes (Figure 1). The magnitude of the NO response to *S epidermidis* CM was significantly lower compared with *S aureus* (Figure 1). The average DAF-FM fluorescence increase was 165.1 ± 50.63 for *S aureus* CM and 94.111 ± 25.10 for *S epidermidis* CM ($p = 0.0237$).

Coagulase-negative staphylococci clinical isolate CM elicits a weaker epithelial NO response than *S aureus* clinical isolates

After determining that CM from lab strains of *S epidermidis* and *S aureus* elicit differential NO responses, we investigated whether clinical isolates of coagulase-negative staphylococci and *S aureus* would also show differential NO production. Similarly, we found that the degree of NO production to coagulase-negative staphylococci clinical isolate CM was significantly lower compared with *S aureus* clinical isolate CM (Figure 2). The average DAF-FM fluorescence increases were 170.5 ± 26.69 for *S aureus* clinical isolate CM and 92.39 ± 26.37 for coagulase-negative staphylococci clinical isolate CM ($p < 0.001$).

***S epidermidis* CM contains a low-molecular-weight, heat- and protease-stabile product that elicits a NOS-mediated NO response**

To demonstrate that the increase in DAF-FM fluorescence after apical application of *S epidermidis* CM was due to NO and not another reactive oxygen or nitrogen species, ALI cultures were stimulated with *S epidermidis* CM, LB medium, and *S epidermidis* CM in the presence of the NOS inhibitor L-NAME. DAF-FM fluorescence increase for *S epidermidis* CM was significantly greater than LB and was significantly decreased by incubation with L-NAME (Figure 3B). The mean DAF-FM increase for *S epidermidis* CM (94.12 ± 25.10) was approximately 2.5-fold higher than for LB (40.58 ± 18.44 , $p < 0.05$) and *S epidermidis* CM with L-NAME (37.81 ± 7.631 ; $p < 0.05$). The DAF-FM fluorescence increase for *S epidermidis* CM with L-NAME was comparable to that of LB, demonstrating that the *S epidermidis* product(s) secreted in the CM increased DAF-FM fluorescence via a NOS-mediated pathway.

To characterize the active product(s) in the *S epidermidis* CM, we compared the DAF-FM fluorescence increase for *S epidermidis* CM with CM that was dialyzed to remove low-molecular weight (<3.5 kDa) components, boiled to denature proteins, and digested with the protease trypsin (Figure 3B). Dialysis attenuated the DAF-FM response by >2 -fold (42.79 ± 14.58 ; $p < 0.05$), whereas denaturing the protein content of the CM by boiling (100.1 ± 24.48 ; not significant) or enzymatic digestion (93.28 ± 29.38 ; not significant) did not

significantly affect the response. These results indicate that the active product(s) in the *S epidermidis* CM stimulating sinonasal epithelial NO production was a low-molecular-weight, heat-stable molecule. Furthermore, as proteins with secondary or tertiary structure would most likely be denatured by heat and peptide bonds would be cleaved by a protease, the retained activity suggests that the active product is likely not a polypeptide. It is worth noting that trypsin was also tested independently at the same concentration and had no effects on NO production (not shown).

***S epidermidis* CM–induced NO generation is independent of the T2R-taste-signaling pathway**

We next sought to determine whether the NO response to *S epidermidis* CM was T2R-independent, as seen with *S aureus*,¹² or T2R-dependent as previously demonstrated by *P aeruginosa* AHLs.¹¹ We found that the NO generation was not blocked by an inhibitor of PLC β -2, which activates Ca²⁺ signaling downstream of bitter taste receptors. The average DAF-FM fluorescence increases in cultures were 89.85 ± 23.75 with *S epidermidis* CM alone, 83.00 ± 17.18 in the presence of the PLC β -2 inhibitor U73122, and 91.51 ± 37.87 in the presence of its inactive analog U73343 (Figure 4A).

Similarly, we found that the presence of an inhibitor of the TRPM5 ion channel, another canonical component of T2R signaling, had no significant effect on the NO response to *S epidermidis* CM. The average DAF-FM fluorescence of cultures stimulated with *S epidermidis* CM alone or in the presence of TPPO (*S epi* CM + TPPO) was 89.85 ± 23.75 and 88.29 ± 29.30 , respectively ($p = 0.9319$; Figure 4B). Together, the results for the PLC β -2 and TRPM5 inhibitors strongly suggest that NO generation is not specific to the T2R pathway and is more similar to the response seen with the gram-positive *S aureus* than the gram-negative *P aeruginosa*.

Discussion

This is the first study to demonstrate NOS-mediated NO production in differentiated sinonasal epithelial cells in response to *S epidermidis* product(s). This host-pathogen signaling and NO production was similar, but less robust than the response to *S aureus* compared with our previous observations.¹² *Staphylococcus* species have often been described as chronic colonizers of the upper airway; however, there is widespread debate about what factors or alterations of the sinonasal microbiome predispose to chronic upper airway disease versus symbiosis between host and pathogen.^{13–15,17} Although both *S aureus* and *S epidermidis* are commonly isolated from sinonasal cultures of chronic rhinosinusitis patients,^{3,18,19} coagulase-negative staphylococci like *S epidermidis* are often considered nonpathogenic, although this distinction is controversial.^{20,21}

NO is an integral component of upper airway defense because it increases mucociliary clearance and has antimicrobial effects,^{5,6} including the ability to disrupt biofilms,²² which are commonly produced by airway bacteria such as *S epidermidis*.²³ Our prior work showed the direct bactericidal effects of NO produced by sinonasal ALI cultures in vitro,¹¹ as demonstrated in this study. Herein we have demonstrated that the intracellular NO response to *S epidermidis* is rapid, as seen with *S aureus*; however, the NO generation plateaued more

quickly with *S epidermidis* product(s), lasting about 2 minutes compared with about 5 minutes with *S aureus*. It is possible that the differential response between *S aureus* and *S epidermidis* is related to a more efficient human innate immune response to more virulent organisms. Moreover, these results may indicate that nonpathogenic colonizers of the airway, including coagulase-negative staphylococci and specifically *S epidermidis*, elicit a low-level response that is adequate enough to prevent epithelial damage, biofilm formation, and invasion in healthy individuals.

Secreted bacterial products called quorum-sensing molecules are small, diffusible molecules that bacteria use for communication, control of gene expression, formation of biofilms, antibiotic production, and expression of various virulence factors. AHLs are the quorum-sensing molecules used by gram-negative bacteria, whereas gram-positives more commonly use small peptides for signaling.^{24,25} In this study and our prior work, we demonstrated that (a) product(s) is/are secreted into the growth media by the gram-positive organisms *S aureus* and *S epidermidis* that is likely not a peptide and is <3.5 kDa in size. This conclusion was made because a protein with secondary or tertiary structure would likely be disrupted by boiling and thus lose activity; similarly, the activity of a peptide would be expected to be diminished after treatment with the protease trypsin, neither of which were seen with the staphylococcal product(s). It is possible, but less likely, that the active product(s) is a small peptide resistant to heat denaturing that also does not contain arginine or lysine sites, where trypsin cleaves.

Within the confines of both studies, it appears that the *S aureus* and *S epidermidis* product(s) have many similar characteristics and may have identical compositions. It is possible that *S aureus* and *S epidermidis* produce similar products at a different rate or that they produce entirely different products with varying efficacies in activating epithelial NO production. Regardless, further studies are required to explore the specific composition of the active product(s) and determine their potential role in bacterial quorum-sensing.

As in our previous study with *S aureus*,¹² we demonstrated that the NOS-mediated NO response to *S epidermidis* is independent of T2Rs such as T2R38, which is known to activate NO production by NOS in response to gram-negative AHL quorum-sensing molecules.¹¹ It will be necessary to determine the specific receptor/pathway responsible for binding the *S epidermidis* products and whether such a receptor responds to various *Staphylococcus* species/products. Because insufficient sinonasal innate immunity predisposes to chronic rhinitis,^{4,26,27} identification of a receptor that can stimulate epithelial NO production in response to *Staphylococcus* could provide a novel target for pharmacologic manipulation and substitute for current therapies and antibiotics.

Conclusion

In this study we have described a NO-mediated innate defense pathway in upper airway epithelium that responds to product(s) secreted by *S epidermidis*. The active product is likely a small, nonpeptide molecule that may be shared among *Staphylococcus* species and is able to trigger an epithelial NO pathway independent of bitter taste receptors. The heterogeneous response to different *Staphylococcus* species may be related to the pathogenicity of different

bacteria and may contribute to the multifactorial nature of chronic rhinosinusitis. Identification of the active product and human receptor(s) will enhance the putative therapeutic targets to combat airway infectious processes.

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FIGURE 1.

Epithelial cell NO response is more robust to *S. aureus* than *S. epidermidis* CM. (A) Representative traces of DAF-FM fluorescence for *S. aureus* CM and *S. epidermidis* CM. (B) Bar graphs of average DAF-FM fluorescence (n = 4 to 9 cultures; 3 to 9 patients for each condition) of cultures stimulated with *S. aureus* CM or *S. epidermidis* CM. DAF-FM fluorescence increases were 165.2 ± 50.63 (*S. aureus* CM) vs 94.112 ± 25.10 (*S. epidermidis* CM; $p = 0.0237$). Graph shows mean $\pm$ SD. * $p < 0.05$; CM = conditioned medium; DAF-FM = 4-amino-5-methylamino-2',7'-difluorofluorescein; NO = nitric oxide; *S. epi* = *Staphylococcus epidermidis*.

FIGURE 2.

Epithelial cell NO production is stronger in response to *S aureus* clinical isolate CM than coagulase-negative staphylococci clinical isolate CM. Average DAF-FM fluorescence (n = 8 or 9 clinical isolates; 3 patients for each condition) of cultures stimulated with *S aureus* clinical isolate CM (*S. aureus* CI CM) or coagulase-negative staphylococci clinical isolate CM (CNS CI CM). DAF-FM fluorescence increases were 170.5 ± 26.69 (*S. aureus* CI CM) vs 92.39 ± 26.37 (CNS CI CM; $p = 0.0237$). Graph shows mean $\pm$ SD. ** $p < 0.01$. CM = conditioned medium; CI = clinical isolates; DAF-FM = 4-amino-5-methylamino-2',7'-difluorofluorescein; NO = nitric oxide; SD = standard deviation.

FIGURE 3.

S. epidermidis CM induces NOS-mediated NO production through a low-molecular-weight, heat- and protease-stable product. (A) Representative traces of DAF-FM fluorescence for *S. epidermidis* CM, LB, *S. epidermidis* CM + L-NAME (NOS inhibitor), dialyzed *S. epidermidis* CM (in LB, 3.5 kDa cutoff, 5 hours), boiled *S. epidermidis* CM (100°C, 1 hour), and *S. epi* CM digested with trypsin (250 µg/mL, 37°C, 1 hour). (B) Bar graphs of average DAF-FM fluorescence (n = 3 or 4 cultures; 3 or 4 patients for each condition) of cultures stimulated with *S. epidermidis* CM, LB, *S. epi* CM + L-NAME, dialyzed *S. epidermidis* CM, boiled *S. epidermidis* CM, and trypsinized *S. epidermidis* CM. DAF-FM fluorescence increases were 94.12 ± 25.10 (*S. epidermidis* CM) vs 40.58 ± 18.44 (LB; $p < 0.05$ by 1-way ANOVA with Bonferroni's posttest) vs 37.81 ± 7.631 (*S. epidermidis* CM + L-NAME; $p < 0.05$) vs 42.79 ± 14.58 (dialyzed *S. epidermidis* CM; $p < 0.05$) vs 100.1 ± 24.48 (boiled *S. epidermidis* CM; ns) vs 93.28 ± 29.38 (trypsinized *S. epidermidis* CM; ns). Graph shows mean ± SD. * $p < 0.05$. CM = conditioned medium; DAF-FM = 4-amino-5-methylamino-2', 7'-difluorofluorescein; LB = lysogeny broth; L-NAME = L- N^G -nitroarginine methylester; NOS = nitric oxide synthase; ns = not statistically significant; *S. epi* = *Staphylococcus epidermidis*; SD = standard deviation.

FIGURE 4.

S. epidermidis CM-induced NO generation is not blocked by inhibitors of PLC β -2-dependent Ca²⁺ signaling or inhibitors of TRPM5-dependent Ca²⁺ signaling. (A) Bar graphs of average DAF-FM fluorescence (n = 3 to 5 cultures; 3 patients for each condition) of cultures stimulated with *S. epidermidis* CM alone or in the presence of U73122 (PLC β -2 inhibitor) or U73343 (inactive analog). After stimulation with *S. epidermidis* CM, DAF-FM fluorescence increases were 91.51 ± 37.87 (*S. epidermidis* CM + U73343) vs 89.85 ± 23.75 (*S. epidermidis* CM; ns by 1-way ANOVA with Bonferroni's posttest) vs 83.00 ± 17.18 (*S. epidermidis* CM + U73122; ns). (B) Bar graphs of average DAF-FM fluorescence (n = 4 or 5 cultures; 3 or 4 patients for each condition) of cultures stimulated with *S. epidermidis* CM alone or in the presence of TPPO (TRPM5 blocker) (*S. epidermidis* CM + TPPO). After stimulation with *S. epidermidis* CM, DAF-FM fluorescence increases were 89.85 ± 23.75 (*S. epidermidis* CM) vs 88.29 ± 29.30 (*S. epidermidis* CM + TPPO; *p* = 0.9319). Graph shows mean ± SD. ANOVA = analysis of variance; CM = conditioned medium; DAF-FM = 4-amino-5-methylamino-2',7'-difluorofluorescein; ns = not statistically significant; *S. epi* = *Staphylococcus epidermidis*; SD = standard deviation; TPPO = triphenylphosphine oxide; TRPM5 = transient receptor potential melastatin isoform 5.