Inhibition of Otopathogenic Biofilms by Organoselenium-Coated Tympanostomy Tubes

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**IMPORTANCE**  Tube occlusion and post–tympanostomy tube otorrhea (PTTO) are 2 major sequelae of tympanostomy tube placement. Plugging negates the function of the tympanostomy tubes and, along with chronic PTTO, can be financially burdensome owing to repeated surgical procedures and additional treatments.

**OBJECTIVE**  To investigate the effectiveness of an organoselenium (OSe) coating on Donaldson tympanostomy tubes in inhibiting biofilm formation on the tympanostomy tubes.

**DESIGN**  In vitro microbiologic study; all experiments were performed in a Texas Tech University Health Sciences Center basic sciences laboratory.

**INTERVENTIONS**  Inhibition of biofilm formation was investigated by incubating OSe-coated vs uncoated (control) tympanostomy tubes in a nutrient broth containing either *Staphylococcus aureus* (*Sa*) expressing green fluorescent protein (GFP), nontypeable *Haemophilus influenzae* (NT*Hi*) expressing GFP, or *Moraxella catarrhalis* (Mc) for 48 hours at 37°C. All biofilms were quantified via colony-forming unit (CFU) assays. The *Sa* and NT*Hi* biofilms were visualized using confocal laser-scanning microscopy (CLSM) and analyzed using the COMSTAT program.

**MAIN OUTCOMES AND MEASURES**  The CFU assays, CLSM, and COMSTAT analysis revealed that compared with uncoated control tympanostomy tubes, OSe-coated tympanostomy tubes are able to inhibit *Sa*, NT*Hi*, and Mc biofilm formation.

**RESULTS**  The *Sa* and NT*Hi* developed thick mature biofilms containing considerable biomass on uncoated tympanostomy tubes as determined by CLSM and COMSTAT analysis, while the OSe coating on the tympanostomy tubes drastically inhibited biofilm formation by *Sa* and NT*Hi*. Quantitative CFU analysis revealed that this reduction in biofilm formation was significant, 6 logs for *Sa* (*P < .001*) and 4 logs for NT*Hi* (*P = .02*). OSe coating also inhibited biofilm formation by Mc with a 4.5-log reduction (*P < .001*).

**CONCLUSIONS AND RELEVANCE**  The OSe coating is a potential long-lasting agent to prevent biofilm development on tympanostomy tubes by otopathogens.
chronic otitis media continues to be a major problem in the pediatric population, necessitating surgical intervention and the placement of tympanostomy tubes. The 2 major problems associated with tympanostomy tube placement are tube occlusion and post–tympanostomy tube otitis media (PTTO).\textsuperscript{1,2} The incidence of tympanostomy tube occlusion ranges from 7\% to as high as 37\%.\textsuperscript{3-4} Many cases of tympanostomy tube plugging require additional surgical procedures to replace the occluded tympanostomy tube. Similarly, PTTO, which occurs at a rate of 10\% to 74\%, requires prolonged and expensive treatments.\textsuperscript{7} Among the different factors that contribute to PTTO are viral and bacterial infections of the upper respiratory tract. Bacterial pathogens that are commonly associated with PTTO are \textit{Staphylococcus aureus} (hereinafter, \textit{Sa}), \textit{Haemophilus influenzae} (\textit{Hi}), \textit{Moraxella catarrahalis} (\textit{Mc}), \textit{Pseudomonas aeruginosa}, and \textit{Streptococcus pneumoniae}.\textsuperscript{5}

The most severe challenge that clinicians face in treating bacteria-related PTTO is biofilm development by these otopathogens.\textsuperscript{6} Biofilms are single-species or multispecies microbially derived sessile communities in which bacteria attach to a substrate or to each other.\textsuperscript{7} Within the biofilm, bacteria are surrounded by a glycocalyx that contributes to the enhanced resistance of the bacteria to host immune responses as well as different antibiotic treatments. The glycocalyx is formed from extrapoly saccharide matrix that is produced by the micro-organisms and the host’s surrounding tissues.\textsuperscript{7} In addition to the protection provided by the biofilm, some pathogenes, such as \textit{Sa} and \textit{P aeruginosa}, are highly resistant to most available antibiotics.\textsuperscript{8,9} Therefore, it is essential to prevent the development of bacterial biofilms on tympanostomy tubes.

Previous reports described strategies designed to inhibit the development of bacterial biofilms on tympanostomy tubes. Among these strategies are antibiotic-coated tympanostomy tubes,\textsuperscript{9} silver oxide–impregnated tympanostomy tubes,\textsuperscript{10-12} polyvinylpyrrolidone-coated silicone tympanostomy tubes,\textsuperscript{13} phosphorylcholine antibacterial coating,\textsuperscript{10,14} and ion-bombarde d silicone and fluoroplastic tympanostomy tubes.\textsuperscript{15} Each one of these strategies has its own limitations. Antibiotic coatings are likely to cause an increase in antibiotic resistance; silver oxide–impregnated and phosphorylcholine antibacterial-coated tympanostomy tubes do not inhibit biofilm formation; and ion-bombar dediated tympanostomy tubes do not completely inhibit bacterial attachment.

Selenium occurs naturally as inorganic forms (Se\textsuperscript{2-}, SeO\textsubscript{2}\textsuperscript{2-}, SeO\textsubscript{2}\textsuperscript{2-}) and organic compounds, such as dimethyl selenide and selenocysteine, and is stable in air and water. Organo selenium (OSe) is a compound that is capable of catalyzing the formation of superoxide radicals: 2 R-SH + R-Se\textsuperscript{2-} + 2 O\textsuperscript{2-} \rightarrow R-Se\textsuperscript{2-} + 2 O\textsuperscript{2-} + R-SH + 2 H\textsuperscript{2}.\textsuperscript{16-37} Superoxide radicals lead to the production of hydroperoxyl radicals, a neutral species that can enter the bacterial membrane leading to inhibition of bacterial colonization of solid surfaces: \textit{O}_{2}\textsuperscript{•-} + H\textsuperscript{2} \rightarrow HO\textit{O}_{2}\textsuperscript{•-}.\textsuperscript{17} Organoselenium coatings have been successfully used to prevent the development of \textit{Sa} biofilms on hemodialysis catheters.\textsuperscript{18} In this study, we investigated the effectiveness of a novel OSe coating on Donaldson tympanostomy tubes in inhibiting biofilm formation by the otopathogens \textit{Sa}, nontypeable (NT) \textit{Hi}, and \textit{Mc} on the tympanostomy tubes.

Methods

Bacterial Strains and Culture Conditions

The pathogenic bacterial strains utilized in this study were (1) \textit{Sa} strain AH133, which carries plasmid pCM11 for expression of green fluorescent protein (GFP)\textsuperscript{5}; (2) \textit{NTHi} strain 86-028NP, which carries the plasmid pRSM2211 that also encodes GFP\textsuperscript{20}; and (3) \textit{Mc} strain 43617, which was purchased from the American Type Culture Collection. The \textit{NTHi} strain was originally obtained from a child with chronic otitis media.\textsuperscript{20}

For general growth, the strains were grown in the following media and under the following conditions. \textit{Sa}-AH133 was grown aerobically at 37\^\circ C in Luria-Bertani broth, Mc-43617 was grown aerobically at 37\^\circ C in brain heart infusion (BHI) broth, and \textit{NTHi}-86-028NP was grown in BHI broth supplemented with nicotinamide adenine dinucleotide and heme (sBHI) to a final concentration of 2 μg/mL at 37\^\circ C with 5\% carbon dioxide.\textsuperscript{20} Biofilm development was examined using sBHI for \textit{NTHi}-86-028NP, BHI for \textit{Mc}-43617, and trypticase soy broth in a 1:25 dilution of 1X phosphate buffered saline (PBS) (dTSB) for \textit{Sa}-AH133. Erythromycin. 1 μg/mL, or kanamycin, 20 μg/mL, was added to the growth medium to maintain the GFP plasmids in \textit{Sa}-AH133 and \textit{NTHi}-86-028NP, respectively.

Coating the Tympanostomy Tubes With OSe

Uncoated silicone Donaldson tympanostomy tubes with a 1.14-mm inner diameter were purchased from Medtronic Xomed. To coat the tympanostomy tubes with OSe, toluene was first added to the tympanostomy tubes, and the tympanostomy tubes were heated to 90\^\circ C. Seldox, which is a methacylate containing covalently attached selenium, was then added followed by a thermal free radical initiator. The percentage of selenium in the Seldox is 35.55\%. The concentration of Seldox used is 0.25\% by weight for the coating solution. Thus, the selenium concentration in the coating is 0.09\% by weight. The addition of Seldox and the radical initiator was repeated twice. In each case, a yellow color developed in the solution and then gradually disappeared, indicating that the reaction was complete. The tympanostomy tubes were then rinsed with absolute ethanol first to remove any organic residues and several times after with distilled water. The presence of the selenium reduction-oxidation activity on the coated tympanostomy tubes was confirmed using the chemiluminescence assay. We excluded the possibility that other manipulations (other than the addition of Seldox) inhibit the development of bacterial biofilms. We prepared control tympanostomy tubes that were subjected to the treatments described herein, except for the addition of Seldox. Control tympanostomy tubes did not interfere with the development of bacterial biofilms (data not shown). The OSe-coated tympanostomy tubes were sterilized by ethylene oxide sterilization prior to use.
In Vitro Static Biofilm Assay

The assay was conducted as described with some modification. Sterile biofilm medium (dTSB, sBHI, or BHI, as appropriate) was aliquoted into each well of a sterile 96-well polystyrene microtiter plate (Costar; Corning). Each tympanostomy tube was removed from its sterile package and placed into individual wells. Overnight cultures of Sa-AH133, NTHi-86-028NP, or Mc-43617 were pelleted, resuspended, and diluted in sterile 1X PBS. The diluted cultures were inoculated into the medium containing the tympanostomy tubes to initial optical densities of samples measured at a wavelength of 600 nm of 0.42, 0.22, and 0.22, respectively. The plates were then covered and incubated at 37°C for 48 hours with slight shaking (250 rpm) using a titer plate shaker (Lab-Line Instruments). After 48 hours, tympanostomy tubes were carefully removed and rinsed twice in sterile 1X PBS to remove any planktonic bacteria. Biofilms on the tympanostomy tubes were either quantified or visualized. In addition, planktonic cells in the spent medium from the Sa biofilm assay were processed to determine the effect of OSe-tympanostomy tubes on the growth of planktonic cells.

Quantification of the Biofilm

Each rinsed tympanostomy tube was placed into a 1.5-mL microcentrifuge tube containing 1 mL of 1X PBS. The tympanostomy tubes were then vigorously vortexed to disrupt the biofilm and detach bacterial cells. The suspended cells were then serially diluted 10-fold in 1X PBS, and 10 μL aliquots of each dilution were spotted onto Luria Bertani agar plates for Sa-AH133 or chocolate agar plates for Sa-AH133 or chocolate agar plates for NTHi-86-028NP and Mc-43617. The plates were incubated at 37°C for 24 hours, and the numbers of micro-organisms (colony forming units [CFUs]) were counted. The CFUs per tympanostomy tube were determined using the following formula: CFUs × Dilution Factor × 100. Each experiment was repeated 3 times for reproducibility. Similarly, the planktonic cells within the spent medium from the Sa biofilm assay were serially diluted, and the CFUs per milliliter were determined.

Confocal Laser Scanning Microscopy

Biofilms formed by Sa or NTHi were visualized by confocal laser scanning microscopy (CLSM) using an Olympus IX71 Fluoview 300 confocal laser scanning microscope (Olympus America). Each rinsed tympanostomy tube was longitudinally split to visualize the biofilm on the inner and outer surfaces of the tympanostomy tubes. All images were obtained via a 10X UPlanFL/0.30NA objective using a multiargon laser (488 nm) with 510/530-nm barrier filters. Three-dimensional biofilm image reconstructions were performed using NIS-Elements 2.2 software (Nikon Instruments).

Structural Analysis of the Biofilm

Structural analysis was performed using the COMSTAT program as previously described. Multiple slices (72-299 depending on the depth of the biofilm) of each biofilm were used to reconstruct the 3-dimensional images. A representative image stack was obtained from a random position within each biofilm for each experiment. The image stacks were examined for the following structural features: biomass or volume of the biofilm (μm³/μm²); average thickness (μm); surface area (μm²), a reflection of the efficiency with which the strain colonizes the surface; and surface area to biomass ratio, an estimate of the portion of the biofilm exposed to nutrients.

Statistical Analysis

Differences in viable biomass (in CFUs) between strains grown on OSe-coated and uncoated tympanostomy tubes were analyzed by unpaired 2-tailed t tests using GraphPad Prism, version 4.03.

Results

Inhibition of Sa Biofilm Development on Tympanostomy Tubes by OSe Coating

Before analyzing the ability of OSe coating on tympanostomy tubes to inhibit biofilm formation by otopathogens, we first determined whether the bacteria would form biofilms on Donaldson tympanostomy tubes, and if, due to the narrow lumen of the tympanostomy tubes, a more robust biofilm would develop on the inner surface rather than on the outer surface of the tympanostomy tubes. To address these questions, Sa-AH133 was incubated with uncoated tympanostomy tubes in the microtiter plate biofilm assay system. The tympanostomy tubes were split longitudinally, and the biofilms formed on the inner and outer surfaces of the tympanostomy tube were visualized by CLSM. Sa-AH133 developed biofilm on both surfaces of the tympanostomy tubes, with that on the inner surface appearing denser and more fully developed than the outer surface biofilm (Figure 1A and B). To confirm that these structures represent biofilm and not simple colonization of the tube surfaces with Sa-AH133, we performed a 3-dimensional analysis. As shown in Figure 1C and D, Sa-AH133 formed a mature biofilm on the inner surface of the tympanostomy tubes that is visibly thicker than the biofilm developed on the outer surface.

We then compared the extent of biofilm development by Sa-AH133 on uncoated tympanostomy tubes and tympanostomy tubes coated inside and outside with OSe. Quantification of the total number of CFUs per tympanostomy tube (bacteria on both the inner and outer surfaces of the tympanostomy tube) revealed a significant reduction of 6 logs (P < .001) in the CFUs of Sa-AH133 present on the OSe-coated tympanostomy tubes compared with the uncoated tympanostomy tubes (Figure 2A). Visualization by CLSM as well as 3-dimensional analyses confirmed these results. Sa-AH133 formed biofilms on both surfaces of the uncoated control tympanostomy tubes with a thicker and more developed biofilm on the inner surface (Figure 3). To confirm these findings, we evaluated different features of the biofilm using the COMSTAT program. Compared with the inner surface biofilm, the total biomass, average thickness, and surface area of the biofilm on the outer surface were considerably reduced (Table). In contrast, we detected no biofilm on either surface of the OSe-coated tympanostomy tubes (Figure 3). In fact, Sa-AH133 even failed to efficiently colonize the surface of the tympanostomy tubes. Only
afewscatteredcellswere detected (Figure 3). Theseresultssug-
gest that OSecoatingoftympanostomytubesinhibitsthede-
velopment of Sa-
AH133 biofilm ontheir surfaces.

Inhibition of Hi and Sa Biofilm Development on
Tympanostomy Tubes by OSe Coating
Two other pathogens that contribute to otitis media with ef-
fusion are Hi and Mc. We quantified and visualized NTHi-86-
028NP biofilm using CFU analyses and CLSM. Similarly to Sa-
AH133, NTHi-86-028NP produced biofilm on both sides of the
uncoated control tympanostomy tubes, with the biofilm on the
outer surface far less developed than that on the inner sur-
face (Figure 3). COMSTAT analyses of different features of the
biofilm confirmed these results (Table). Compared with the un-
coated tympanostomy tubes, the total CFUs recovered from
the inner and outer surfaces of the OSe-coated tympanos-
tomy tubes were significantly lower (P = .02) (Figure 2B). In ad-
dition, CLSM revealed only a few individual micro-organisms
on either surface of the OSe-coated tympanostomy tubes (Figure 3). Furthermore, and in contrast to uncoated control tympanostomy tubes, COMSTAT analyses revealed no biofilm features on the OSe-coated tympanostomy tubes (Table). This trend in the ability of the OSe coating to inhibit biofilm formation by otopathogens continued with Mc. Quantitative analysis of biofilms formed by Mc-43617 showed that the strain produced a substantial biofilm on uncoated control tympanostomy tubes. However, it failed to develop a biofilm on the OSe-coated tympanostomy tubes, with a 4.5-fold reduction

<table>
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<tr>
<th>Bacterial Strain</th>
<th>Location and Treatment</th>
<th>Biomass, μm³/μm²</th>
<th>Thickness, Mean, μm</th>
<th>Surface Area, μm²</th>
<th>Surface Area/Biomass Ratio</th>
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<tr>
<td>Sa-AH133</td>
<td>Inner surface</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td></td>
<td>Outer surface</td>
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<td>579 082</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>OSe coated</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NTHI-86-028NP</td>
<td>Inner surface</td>
<td>0.12</td>
<td>0.13</td>
<td>426 917</td>
<td>1.85</td>
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<tr>
<td></td>
<td>Outer surface</td>
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<td>285.79</td>
<td>52 732 700</td>
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<td>0.00005</td>
<td>0</td>
<td>240</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Abbreviations: NTHI, nontypeable *Haemophilus influenzae*; OSe, organoselenium; Sa, *Staphylococcus aureus*.

* Reflects the efficiency with which bacteria colonized the tympanostomy tube surface.

* Estimates the portion of the biofilm exposed to nutrients.
Inhibition of Otopathogenic Biofilms

Stability of the Antibiofilm Effect of OSe-Coated Tympanostomy Tubes
An important quality of antimicrobial coatings on medical devices is their shelf stability. To test the shelf stability of OSe coatings, we coated the tympanostomy tubes and stored them in a sterile container for 9 months at room temperature before using them in the biofilm assay with Sa-AH133. OSe-coated tympanostomy tubes stored for 9 months were very effective in inhibiting the development of Sa-AH133 biofilm (P < .001) (Figure 4). Therefore, it is likely that the OSe coating of tympanostomy tubes would remain effectual even after an extended period of storage.

Inhibition of Planktonic Growth by OSe-Coated Tympanostomy Tubes
Biofilm formed on the tympanostomy tubes may initiate middle ear infection by continuously releasing planktonic cells. Alternatively, planktonic cells within the middle ear may colonize the tympanostomy tubes and initiate a biofilm. Besides its antibiofilm effect, the small volume of the middle ear cavity may allow these free-swimming bacteria to come into contact with exposed surfaces of the OSe-coated tympanostomy tubes, resulting in their deaths from the contact. Alternatively, superoxide radicals catalyzed by the OSe coating may diffuse into the limited space and kill or inhibit the bacteria. To test this possibility, we conducted the biofilm assay using Sa-AH133 but determined the number of microorganisms recovered from the dTSB biofilm medium. The number of CFUs of Sa-AH133 within the biofilm medium of OSe-coated tympanostomy tubes was reduced by 7 logs (P < .001) compared with the CFUs in the biofilm medium of uncoated control tympanostomy tubes (Figure 5). This suggests that the OSe coating does inhibit the growth of planktonic cells.

Discussion
Results of this study indicate the effectiveness of OSe coating of tympanostomy tubes in inhibiting biofilm development by otopathogens. Quantitative analysis showed that OSe coating of tympanostomy tubes significantly reduced the tympanostomy tube colonization and biofilm development by Sa, Hi, and Mc (Figure 2). Microscopy, 3-dimensional analyses, and COMSTAT analyses of biofilms formed by Sa-AH133 and NTHi-86-028NP strongly supported the results of the quantitative analysis. The OSe coating inhibited biofilm development on the inner and outer surfaces of the tympanostomy tubes; indeed, only a few individual bacteria managed to attach to either surface (Figure 3).

Numerous previous studies demonstrated that silver oxide–coated medical devices, including urinary catheters, heart valves, and central venous catheters, reduced colonization of bacterial pathogens. Initial analysis indicated the effectiveness of silver oxide–coated tympanostomy tubes in reducing the incidence of otorrhea. However, subsequent scanning electron microscopy (SEM) analysis showed that both untreated and silver-impregnated silicone tympanostomy tubes were susceptible to the development of Sa biofilms. Similarly, Saidi et al showed by in vivo analysis using the guinea pig model of ear infection that Sa adhered to and formed biofilms on both uncoated and silver oxide–coated silicone tympanostomy tubes. All tympanostomy tubes used in this study were silicone Donaldson tympanostomy tubes. Despite the use of silicone, the OSe-coated tympanostomy tubes were very effective in inhibiting the development of Sa biofilm (Figure 2A). Such an effect extended to the other 2 otopathogens, Mc and

<Figure 4. Organoselenium-Coated Tympanostomy Tubes Stored for 9 Months That Inhibited Biofilm Development by Staphylococcus aureus-AH133>

<Figure 5. Organoselenium-Coated Tympanostomy Tubes That Inhibited Planktonic Growth of Staphylococcus aureus-AH133 in Biofilm Medium>
Hi (Figure 2B and C). In addition, even after 9 months of storage, OSe-coated tympanostomy tubes were very effective in preventing the formation of Sa biofilms (Figure 4), indicating a stable coating on both sides of the tympanostomy tubes. Therefore, the novel OSe coating may prove to be more effective in inhibiting bacterial biofilms than the previously described silver oxide–coated or silver-impregnated silicone tympanostomy tubes. Additional experiments, including direct comparison of the antibiofilm effects of OSe-coated tympanostomy tubes and silver oxide–coated tympanostomy tubes, are required to confirm such a hypothesis.

Besides the coatings, the material of the tympanostomy tubes influence the tympanostomy tubes antibacterial effect. Aside from silicone, tympanostomy tubes are made from fluoroplastic. The SEM analyses revealed that compared with fluoroplastic tympanostomy tubes, silicon tympanostomy tubes are more susceptible to bacterial adhesion.24 Among the potential factors that may contribute to this difference are the rough surface texture and the high surface energy of silicone tympanostomy tubes compared with those of fluoroplastic tympanostomy tubes.24 Berry et al10 examined the development of Sa biofilm on uncoated and silver oxide–impregnated fluoroplastic tympanostomy tubes. While no biofilm was detected on the uncoated tympanostomy tubes, evidence of biofilm formation on the silver oxide–impregnated tympanostomy tube was detected.10 The SEM analysis revealed the presence of silver particles on the dry silver-coated fluoroplastic tympanostomy tubes but not on ones that had been soaked in TSB medium.10 This suggests that in solution, silver leaches out of the silver-impregnated tympanostomy tubes, leaving a rough surface that allow Sa to form a biofilm.10 A combination of OSe coating and a fluoroplastic surface may produce a stronger antibiofilm effect. Berry et al10 reported that uncoated fluoroplastic tympanostomy tubes failed to inhibit P aeruginosa biofilms. In preliminary experiments, P aeruginosa biofilms were not as significantly inhibited by the OSe-coated silicone tympanostomy tubes as Sa biofilms (data not shown). However, in coating other medical devices, we showed that incorporating higher amounts of OSe inhibited P aeruginosa biofilm (data not shown). Therefore, it is possible that complete inhibition of P aeruginosa could be achieved by coating the fluoroplastic tympanostomy tubes with higher concentrations of OSe.

To our knowledge, this study represents the first comprehensive analysis designed to examine the development of bacterial biofilm on tympanostomy tubes coated with an antimicrobial agent. Previous studies used SEM analyses to assess the development of bacterial biofilms on silicone tympanostomy tubes coated with silver oxide22,25 or antibacterial agents,9 or on uncoated fluoroplastic tympanostomy tubes.10 In contrast, in our study, we quantified the amount of the biofilm formed on uncoated and coated tympanostomy tubes using the CFU assay as well as visualizing the biofilm on the inner and outer surfaces of the tympanostomy tubes using CLSM. The fluorescent signal produced by GFP can be visualized in CLSM without additional preparation. Therefore, we can visualize the expansion of the same biofilm over several days. In addition, we used the COMSTAT program to examine different features of the biofilm. Such a comprehensive analysis will be helpful for conclusively determining the effectiveness of antimicrobial coatings in future investigations.

Besides inhibiting biofilm development on the tympanostomy tubes, OSe also inhibited the growth of planktonic cells within the vicinity of the coated tympanostomy tubes (Figure 5). There are 2 possible explanations to this observation. It is possible that OSe not only inhibited the bacteria from attaching to and colonizing the surfaces of the tympanostomy tube but also prevented the growth of bacteria within the biofilm solution. The small size of the well in which the biofilm reaction was conducted, which mimics the size of the middle ear space, may have facilitated contact with the OSe-coated surfaces of the tympanostomy tubes. Alternatively, conversion of a significant amount of superoxide to hydrogen peroxide (O2− + H+ → HO2− + O2− + O2 → H2O2), which has a longer half-life (minutes), may have allowed its diffusion into the medium, thus killing the bacteria (Figure 3). The amount of hydrogen peroxide produced from each tympanostomy tube is less likely to have an ototoxic effect. Using a commercially available kit (Quantofix Peroxide 25 Test Sticks; Sigma-Aldrich) we assessed the amount of hydrogen peroxide produced in the solution containing OSe-coated tympanostomy tubes. We examined 6 individual OSe–tympanostomy tubes and confirmed that the amount of hydrogen peroxide produced by each tympanostomy tube was less than 500 ng/mL. Such possibilities would also be clinically relevant as OSe-coated tympanostomy tubes would then be useful not only to prevent biofilm development on the tympanostomy tubes but also function as a treatment by eliminating planktonic cells within the middle ear.

In conclusion, otitis media is one of the most common diagnoses for which children and their families seek medical care. There is increased evidence supporting a role for biofilms in the pathogenesis of otitis media. The OSe coating successfully inhibited the formation of Sa, Hi, and Mc biofilms on Donaldson tympanostomy tubes. In addition, OSe may eliminate ear infection by inhibiting the growth of planktonic cells within the vicinity of the OSe-coated tympanostomy tubes. Whether such inhibitory effects may be extrapolated to other common middle ear pathogens such as Streptococcus pneumoniae and Pseudomonas aeruginosa is under investigation.
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