An Organo-Selenium Coating of Tympanostomy Tubes Inhibits the Development of Bacterial Biofilms

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ABSTRACT

Objectives: 1. To successfully coat a tympanostomy tube with organo-selenium. 2. To determine the effect of a selenium coating on the development of a Staphylococcus aureus biofilm on tympanostomy tubes.

Study Design: Organoselenium compounds have the unique property of being able to be covalently attached to a surface and yet still retain their ability to catalyze the formation of superoxide radicals. These radicals are formed by the donation of an electron to oxygen by the selenium atom. The oxidized selenium atom is then reduced by obtaining an electron from sulfur compounds present in the surrounding media. This completes the catalytic pathway (see Figure 1 below). Sulfur compounds such as glutathione are present in all body fluids and work well in this catalytic mechanism. Thus, in contrast to atoms such as silver, the selenium atom does not have to leave the surface of the biomedical device in order to generate a superoxide radical. The presence of this superoxide radical should then function to inhibit bacterial attachment to the surface.

Methods: Tympanostomy tubes were treated with a pulsed plasma in the presence of an organoselenium compound. The tubes were then incubated with Staphylococcus aureus in nutrient broth for 24 hours to allow for the development of a mature biofilm. Tubes were then removed and examined by scanning electron microscopy.

Results: By scanning electron microscopy and colony forming assays, a complete mature biofilm was found on the control (uncoated) tympanostomy tubes. In contrast no biofilm was found on the selenium coated tympanostomy tubes.

Conclusions: The use of a selenium coating provided an effective barrier to the formation of a bacterial biofilm on a tympanostomy tube.

BACKGROUND

During the last few decades, tympanostomy tubes have become the therapeutic gold standard, and the most widely used treatment for otitis media with effusion (OME). Tympanostomy tube occlusion is the most commonly performed surgical operation among children younger than 15 years. In addition to the treatment of OME and accompanied hearing loss, tympanostomy tubes have also been used in the prevention of recurrent acute otitis media [1]. However, in their complications with tympanostomy tubes, such as purulent otorrhea, discharge, and tympanostomy tube occlusion, are common and can dissipate the function of the tube. The occlusion rate varies between 11 and 25% depending on the tube model and facilities at Texas Tech Univ., Lubbock, TX.

A. Coating of Tympanostomy Tubes: The method of coating was a direct plasma deposition of volatilized diphenyl-diselenide via a proprietary pulsed plasma process. This method can be adjusted to produce different concentrations of selenium on the surface of the implant. Coating was carried out at Aeconald’s laboratories at the University of Texas, Arlington.

B. Biofilm Assays: This was done using the microtiter plate assay [8,9]. This standard assay is commonly utilized to examine bacterial attachment and biofilm initiation by different bacteria [8,10]. We utilized it to determine biofilm development by Staphylococcus aureus. Overnight cultures were diluted in BH to obtain 10^1 colony forming unit (CFU). One ml aliquots of the diluted cultures will then be dispersed in each well of a 96-well micro-titer plate. Several wells contained only diluted BH (negative control). For these initial experiments, we utilized either untreated or selenium coated-tympanostomy tubes. The plates were incubated at 37°C with mild shaking for 24 hours. At 24 hour post inoculation, S. aureus forms a mature developed biofilm [8-10]. The pieces are then removed from the wells, rinsed in Phosphate Buffered Saline (PBS) to eliminate loosely attached planktonic cells and processed for biofilm analysis. After that, each tube was transferred into a 1.5 ml microtube container containing 1 ml of PBS. To detach the biofilms, the tubes were vigorously vortexed for 3 minutes. The bacterial suspension from each tube was then serially [(1:1) diluted in PBS and the dilutions will be plated on nutrient agar plates (using their respective media as described above). Triplicates of 5 ml of each dilution were plated on the agar plates (the drop-method) and the plates incubated at 37°C for 16 hours. The number of CFU/tympanostomy tube were calculated. Six tympanostomy tube were used for each treatment. (See Figure 2 below). Some of this work was carried out at Selenium Ltd’s laboratories in Lubbock, TX.

C. Analysis of biofilm development using scanning electron microscopy: Tympanostomy tubes were rinsed in PBS and fixed by submerging them in 2% glutaraldehyde solution for 16 hours. The dried samples were then mounted for biofilm analysis. The analysis was conducted at the electron microscopy facilities at Texas Tech Univ., Lubbock, TX.

RESULTS

1. Selenium can be permanently attached to the surface of a tympanostomy tube.

2. Selenium attached to the surface of a tube is still catalytic.

3. Selenium coated tympanostomy tubes showed complete inhibition (5 logs) of biofilm formation by S. aureus.

CONCLUSIONS

1. Stability studies will be carried out to determine the length of time the selenium coated tube will remain active.

2. Selenium coated tubes will be tested against clinical isolates.

3. Animal studies will be carried out with suitable animal models.

REFERENCES


FUTURE STUDIES

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Fig. 1. Tympanostomy Tube + Se coating SEM of tympanostomy tubes after 24 hours in a S. aureus broth

Fig. 2 Biofilm analysis schema

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