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Photodynamic Therapy to destroy pneumonia associated microorganisms using external irradiation source

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ABSTRACT

An endotracheal tube (ETT) is required for the management of critically ill, mechanically ventilated patients. Ventilator-associated pneumonia (VAP) affects patients hospitalized in intensive care units; its risk of occurrence is 1% to up 3% for each day of mechanical ventilation. The polymicrobial nature of VAP is established with mixed bacterial-fungal biofilms colonizing the ETT. The microbial interaction enhances the microbial pathogenesis contributing to high indexes of morbidity/mortality. Antimicrobial Photodynamic Therapy (aPDT) could be a suitable therapy for decontamination of oral cavity and ETT at the same time, but the use of a fiber optics inside the ETT seems to not be appropriated since a cannula for secretion aspiration has to be introduced into the ETT to keep it´s lumen. The aim of this study is to proof the concept that an external light source from a LED is capable of reach all areas of the ETT. We use a commercial ETT, 60μM methylene blue (MB), and a 660nm diode laser and calculated the transmission coefficient of light in different situations as only tube, tube with biofilm and biofilm+MB. The results prove that is possible to transmit light through the tube even in the presence of MB and biofilm although a high attenuation of about 60% was measured depending on the tested condition.

Keywords: Ventilator-associated pneumonia, methylene blue, LED, endotracheal tube, light attenuation

1. INTRODUCTION

Ventilator-associated pneumonia (VAP) is considered the most frequent infection in Intensive Care Units and is responsible for high mortality and morbidity rates[1].Studies have shown that a biofilm forms rapidly in the endotracheal tubes (ETT) hours after the intubation process, and this biofilm acts as a continuous reservoir for the contamination of the tracheobronchial tree[2].

The mechanisms implicated in the development of VAP are microaspiration that occurs when the microorganisms present at the secretion move towards the distal portion of the tube accumulating above the ETT cuff, and biofilm that migrates along the ETT cuff and inside the lumen enabling the access to the sterile bronchial tree[3].

For a long time, antimicrobials were the only resources to fight infections; however, their indiscriminate use caused the phenomenon known as microbial resistance[4]. Thus, the search for an innovative and effective alternative became necessary. One possible approach is the antimicrobial photodynamic therapy (aPDT). As aPDT works with oxidative stress there is no reason for, nor evidence of any form of resistance to its mechanism of action since, oxygen is toxic to all living beings to a greater or lesser extent[5].

For the application of aPDT to be successful some factors must be carefully analyzed and they are of extreme importance for the set of therapeutic actions to present effectiveness. These include the photosensitizer presence at the target and in

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close proximity to the cell to be eliminated and perhaps one of the most important issues is the illumination of the target area[6]. In the field of VAP and aPDT Biel et al developed a fiber optics to be inserted into the ETT and proved the effectivity of aPDT in vitro against *Pseudomonas aeruginosas* and MRSA[7].

In our work we are proposing the use of an external LED light source to illuminate the external distal portion of the tube. This work was performed to understand light/tube, light/tube+biofilm, light/tube+methylene blue (MB) and light/tube+biofilm+MB interaction to understand if an external light source would be able to accomplish a uniform irradiation of the tube lumen.

2. MATERIALS AND METHODS

An in vitro study of three new ETTs and three from patients admitted to the ICU of the Hospital São Francisco de Assis, who remained for more than 48 hours under mechanical ventilation with clinical suspicion of VAP was performed.
The project was approved by the Ethics Committee (CAAE 66492217.5.00005494) and the patients or their legal representatives signed the free and informed consent form. As a light source a red LED with a center wavelength of 640nm was used and power was maintained at 80mW.

Tube measurement
Three sterile ETTs (Teleflex Medical,(Montevideo, Uruguay) were analyzed. In each ETT, 5 measurements of light attenuation were carried out using calibrated powermeter (LaserCheck - Coherent, USA) at 610nm corresponding to the MB dimer band and 660nm corresponding to the MB monomer band. After the measurements were taken the mean values were calculated and the tube attenuation coefficient obtained according to the formula:

$$I = I_0.e^{-\mu t}$$

Where, I_0 is the intensity of the incident radiation, I is the intensity of the radiation emerge from the material, t is the thickness of the absorber material and μ is the total linear attenuation coefficient and is related to the probability that the photons are absorbed. Figure 1 demonstrates the light/ETT interaction.

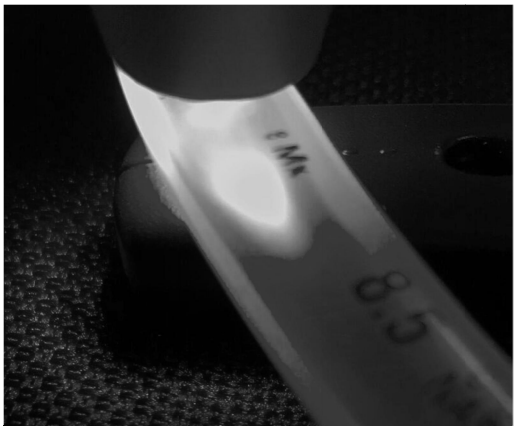

Figure 1 – ETT irradiated with red LED. The figure represents the irradiation of the sterile ETT.

Experimental conditions

Measurements were then carried out following the same methodology proposed above for tubes containing only MB, only contaminated and contaminated and stained with MB. The measurements were performed at the same wavelengths quoted above and the attenuation coefficient for each situation was calculated.

Microscopic analysis

To validate the presence of biofilm an ETT was processed for evaluation by scanning electron microscopy (SEM). Immediately after extubation the tube was cut with a scalpel in the distal 5cm and placed in sterile flasks along with 50ml of 0.9% saline solution. The sample was then washed with 30 ml of saline to remove the mucus and any unbound cells that were not part of the biofilm. Next, a fragment of approximately 2cm was cut and transferred to a sterile flask containing 20ml of 2.0% glutaraldehyde (Rioquímica-São José do Rio Preto, São Paulo) for fixation during 24 hours. After this period, the samples were taken and washed with 30 ml of distilled water and stored in a sterile vial kept in a desiccators until examined.

3. RESULTS AND DISCUSSION

Figure 2 represents the results obtained from the light attenuation in all tested conditions for both wavelengths.

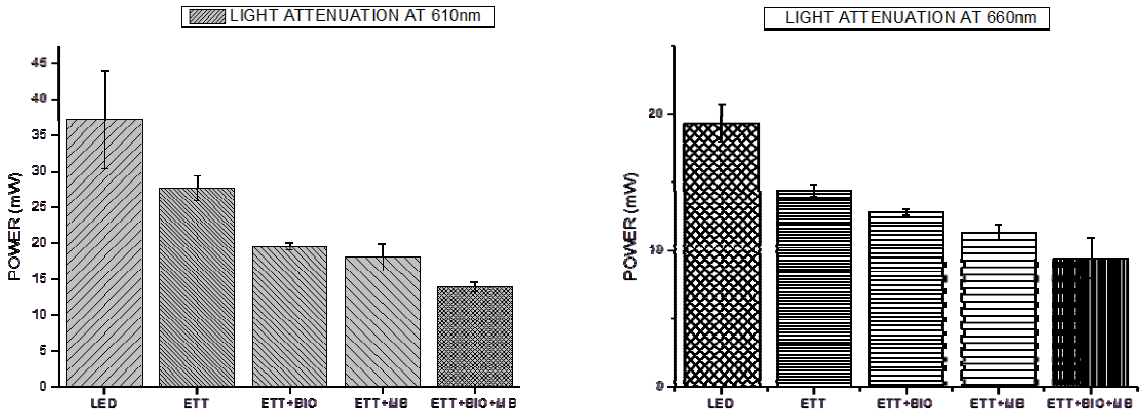

Figure 2 – Light attenuation measurements at maximum absorption of dimer (610nm) and monomer (660nm). LED is the output power at the measured wavelengths, ETT is attenuation through the tube, ETT+BIO is the contaminated tube, ETT+MB is the tube only with MB, and ETT+BIO+MB is the tube contaminated and with MB.

Figure 3 is the graphical representation of the attenuation coefficient calculated for all tested conditions.

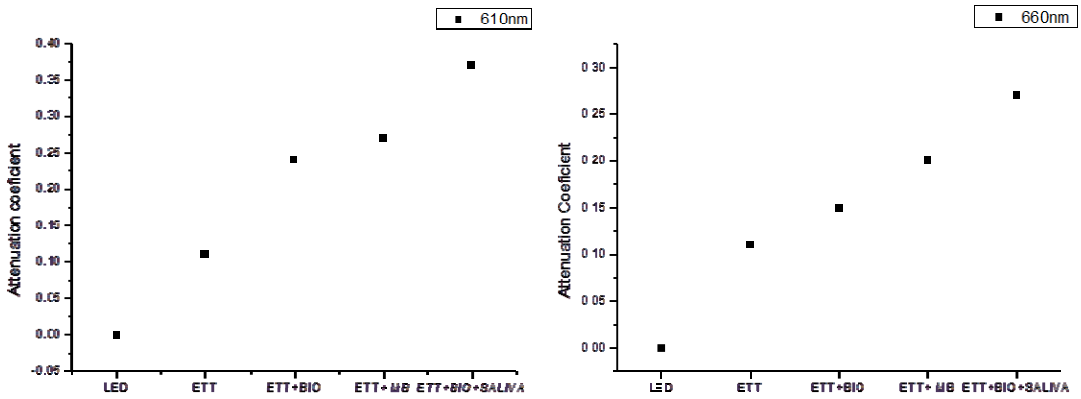

Figure 3 – Calculated attenuation coefficient at maximum absorption of dimer (610nm) and monomer (660nm). LED is the output power at the measured wavelengths, ETT is attenuation through the tube, ETT+BIO is the contaminated tube, ETT+MB is the tube only with MB, and ETT+BIO+MB is the tube contaminated and with MB.

The results demonstrated that although a high attenuation is observed through the ETT there is enough radiation passing across the lumen which would in theory make possible to decontaminate the whole area.

To be certain that a biofilm was present we performed a SEM image of one tube. The image is represented on figure 4.

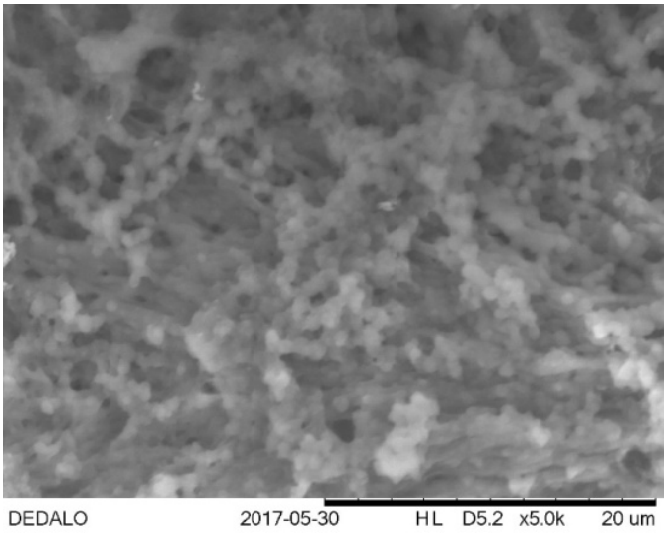

Figure 4 – SEM image obtained from an ETT removed from a patient. We noted an expressive biofilm in the area with extra-cellular matrix and cells.

As both wavelengths may be important for MB photodynamic action we decided to calculate dimer and monomer absorption and as expected the attenuation was similar but with a higher value for the 610nm than 660nm. According to Usacheva et al in the presence of a high number of cells and extracellular slime the dimer is the predominant form of MB therefore our results are in agreement with their results[8].

This study is a proof of concept that it must be possible to use a external light source for ETT decontamination with aPDT and it may be as interesting approach to diminish the risk of VAP. . We will pursuit further investigation to determine the effectiveness of this method

4. CONCLUSION

The results prove that is possible to transmit light through the tube even in the presence of MB and biofilm although a high attenuation of about 60% was measured depending on the tested condition.

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