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Minimum irradiance required to inhibit microbial growth to prevent ventilator-associated pneumonia by an endotracheal tube equipped with blue LEDs

Abstract: Artificial respiration is saving lives especially in the COVID-19 pandemic, but it also carries the risk to cause ventilator-associated pneumonia (VAP). VAP is one of the most common and severe nosocomial infections, often leading to death and adding a major economic burden to the healthcare system. To prevent a proliferation of microbial pathogens that cause VAP, an endotracheal tube (ETT) equipped with blue LEDs (LED-ETT) was developed. This blue wavelength exhibits antimicrobial properties but may also harm human tracheal cells at higher irradiances. Therefore, the aim of this study was to find the minimal required irradiance for microbial reduction of 1 log level in 24 h by applying LED-ETTs. A LED-ETT with 48 blue LEDs (450 nm) was fixed in a glass tube, which served as a trachea model. The investigation was carried out with irradiations of 4.2, 6.6 and 13.4 mW/cm² at 37 °C for 24 h. The experiments were performed with *Acinetobacter kookii* as a surrogate of *Acinetobacter baumannii*, which is classified as critical by the WHO. Samples of *A. kookii* suspensions were taken every 4 h during irradiation from the trachea model. Bacteria concentrations were quantified by determining colony forming units (CFU)/ml. A homogeneous irradiance of only 4.2 mW/cm² generated by the blue LEDs, at a LED forward current of 3.125 mA, is sufficient to achieve a 1 log reduction of *A. kookii* within 24 h. The total irradiation dose within this period was 360 J/cm². Human cells survive this dose without cellular

damage. Previous studies revealed that the pathogen *A. baumannii* is even more sensitive to blue light than *A. kookii*. Therefore, blue LED-ETTs are expected to reduce *A. baumannii* without harming human tracheal cells.

Keywords: LED-ETT, Ventilator-associated pneumonia, VAP, photoinactivation, 450 nm, blue light disinfection, *Acinetobacter kookii*, *Acinetobacter baumannii*

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1 Introduction

An important part of treatment in intensive care units is artificial ventilation by means of an ETT, which is usually applied for days or weeks. An infectious course during hospitalization is called VAP and is one of the most common nosocomial infections [1]. In Europe, approximately 215,000 patients develop VAP annually [2], incurring \$40,000 in additional costs per treatment [3]. The mortality is about 13 % [4]. A major risk that can lead to the development of VAP is colonized endogenous pathogens in oropharyngeal secretions and gastric contents. Aspiration of this pathogens past the larynx into the lower respiratory tract can cause VAP [1]. Pathogens are also introduced through invasive procedures such as the placement of an ETT. With increasing duration of application, a biofilm forms inside the tube as a reservoir for infectious pathogens. Microaspiration is responsible for the influx of pathogens around the ETT cuff into the lower airway, with the tube serving as a gateway [5].

Current treatments, to prevent VAPs, are limited to controlled antibiotic therapy during intensive care and various hygienic preventive measures [6]. Approaches, such as silver coating of the tube [7], have been applied to prevent potential infections, with only antibiotic therapy and hygienic measures proving effective. Another approach is photoinactivation. For

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this purpose, photosensitizers (PS) naturally present in pathogens are irradiated to stimulate the formation of reactive oxygen species (ROS). These damages i. a. the cell walls of the pathogens and they can be inactivated [8]. There also exist PS in the human cells of the trachea, but it was demonstrated that human cells might be less sensitive towards 450 nm light than bacteria. However, this was not tested on epithelial cells but on keratinocytes [9].

In a previous study a prototype was developed for the delivery of light with 450 nm to reduce the risk of VAP by photoinactivation [10]. The LED-ETT with LEDs integrated into the ETT wall proved a general functionality of the prototype against *Staphylococcus carnosus*. Due to safety reasons, it is advantageous to apply currents as low as possible, which however has an impact on the irradiance. But lower irradiances inherit, besides a low current within the body, the additional advantage to protect potentially susceptible human tissue. Due to the low bacterial population in the beginning of an infection, it is assumed that successful treatment does not require a complete bacterial reduction but only an inhibition to reduce or even prevent the growth and spread of pathogens in the area of the ETT. The aim of this study was to investigate the irradiance, which is minimally necessary to reach an inactivation effect of 1 log level within 24 h.

2 Material and Methods

2.1 LED-ETT Prototype and Incubator as thermal test area

For the test setup a LED-ETT prototype [10] is employed. It is equipped with 48 LEDs at 450 nm, which are arranged in a spiral around the tube, with 6 LEDs in series at 8 parallel strings. This results in the best possible balance between minimum current and voltage, at maximum irradiance. The emitted wavelength of the LEDs is 450 nm and irradiances of 4.2 mW/cm² at 25 mA total, 6.6 mW/cm² at 40 mA total and 13.4 mW/cm² at 80 mA total at approximately 16 V are applied during the experiment. The experiments are performed at the human body temperature of 37 °C in an incubator, specifically designed and constructed for this purpose (see Fig. 1) [11].

During operation, the LED-ETT is placed in a glass tube with an inner diameter of 30 mm and a length of 100 mm (see Fig. 2), which thus serves as an idealized trachea. The LED-ETT is connected to the ventilator Servo Ventilator 900C from Siemens (Erlangen, Germany) by means of a standard connector at the upper end. A test lung from Siemens (Erlangen, Germany) at the lower end of the glass tube is the

Figure 1: Schematic setup with LED-ETT, incubator, medical ventilator, laboratory power supply (operates the light emission of the LEDs), glass tube (simulates the trachea), test lung and bacterial solution for microbiological assessment.

Figure 2: LED-ETT inside a glass tube representing the trachea. The cuff is sealing the tube and bacterial solution mimicking the patient's secretion is filled in the glass trachea. LEDs are not powered (a) and powered (b).

counterpart for proper operation. For the irradiation experiments, the space on top of the cuff, between the ETT and the glass tube, is filled with a bacterial suspension, which simulates the patient's secretion (see Fig. 2). The temperatures in the bacterial solution, ETT and incubator are monitored with a digital thermometer type UNI-T UT325 from Uni-Trend (Kowloon, Hong Kong) at each sampling.

2.2 Microbiological Experiments

The experiments are carried out in a S1 laboratory. Therefore, the application of pathogen *Acinetobacter baumannii* is restricted. *A. kookii* (DSM 29071) is the chosen surrogate as it exhibits similar biochemical characteristics [12]. Further, this strain was demonstrated before to be comparably sensitive to visible light irradiation of 405 and 450 nm as its pathogen relative *A. baumannii*. *A. kookii* is cultured in DSMZ 220 (Merck 105458) medium. The liquid medium contains 15.0 g of Peptone from casein, 5.0 g of Peptone from soymeal and 5.0 g of Sodium chloride per 1000 ml distilled water. It is adjusted to pH 7.3. To obtain a preculture 3 ml DSMZ 220 medium is inoculated with one colony of an *A. kookii* agar plate and cultivated for 16 h at 37 °C in a shaking incubator at 170 rpm. Next, for the main culture, 30 ml of DSMZ 220 is prepared and inoculated with 200 µl of the preculture. After approximately 4 h at 37 °C in a shaking incubator at 170 rpm the main culture reaches an optical density of $OD_{600nm} = 0.31$. The *A. kookii* test solution has a bacterial concentration of 10^7 CFU/ml. The main culture is centrifuged at 7,000 rpm for 5 min. The medium is discarded, and the pellet resuspended in phosphate buffered saline (PBS). After one washing steps, the bacterial concentration is adjusted to 10^6 CFU/ml with PBS. The final volume of the test solution for the irradiation experiment results in 60 ml. For the test, 45 ml of the bacterial test solution are placed in the glass tube until all LEDs are covered (see Fig. 2). The remaining 15 ml are kept light protected in the incubator as a control without irradiation. One experiment is set to 24 h, which is assumed to be the minimum treatment duration for patients with artificial respiration. During this 24 h period, every 4 h a sample is taken of approximately 1 ml from the test trachea with LED-ETT and from the control using a sterile-packed disposable Pasteur pipette. The sample after either 12 or 16 h is skipped due to overnight testing. The test solution is homogenized with the pipette prior to sampling, to avoid possible sedimentation of bacteria on the cuff surface. Dilution series are prepared according to the appropriate concentrations. The samples are plated on DSMZ 220 agar for the dilutions in triplicates. All plates are incubated at 37 °C for 24 h and enumerated manually. The determined counts for CFUs are converted to log reductions. Each experiment for the different irradiances was repeated independently at least three times.

3 Results

The temperature of the incubator, the bacterial solution and inside the ETT were measured every 4 h. The operating

temperature of the incubator over 24 h ranged between 36.5 and 37.3 °C. The temperature of the bacterial solution was running close to that of the incubator. The temperature inside the ETT was 1.4 to 2.4 °C lower than that of the incubator, which is probably due to a heat dissipating air flow in the form of the ventilation air. The results of the 24 h irradiation experiments with irradiances of 4.2, 6.6 and 13.4 mW/cm² are illustrated in Figure 3 as a function of time. An overview of the required irradiation dose for a reduction by 1 log level, as well as the required time, depicted given in Table 1.

The time course at the applied low irradiances reveals that a start-up time is required before the photoinactivation effect starts. This is known as the so-called shoulder effect, which is explained by the necessity to overcome bacterial defenses.

Figure 3: Irradiance of 4.2, 6.6 and 13.4 mW/cm². Mean values of at least three independent experiments are plotted over the irradiation duration with error bars indicating the standard deviation of the individual measurements. The dashed lines illustrate the target value range of 1 log level reduction.

Table 1: Values for time and dose, which are necessary to achieve 1 log level reduction of *A. kookii* with a 450 nm LED-ETT at different irradiances.

Irradiance [mW/cm ²]	Time [h]	Irradiation dose [J/cm ²]
4.2	16	240
6.6	12	285
13.4	10	460

4 Discussion

The plotted results in Figure 3 demonstrate that high irradiance is not necessary to inhibit the growth of pathogens. Thus, with an irradiance of 4.2 mW/cm² a reduction of at least 1 log level

is achieved in 24 h, which is assumed to be an inhibition. With the LED-ETT application time of one day, the intended effect was reached even with a lower radiation dose. Considering that ventilation is a treatment over several days, the irradiation regarding *A. kookii* can be performed at 4.2 mW/cm² to minimize the stress on the surrounding tissue. The results of Hoenes et al. [13] reveal a stronger photoinactivation for *A. baumannii* than for *A. kookii* starting at about 150 J/cm². Thus, the irradiation dose of 240 J/cm² in 24 h with 4.2 mW/cm² should be sufficient to achieve at least a similar inhibitory effect for *A. baumannii*. Consequently, this value can be seen as a benchmark for the testing on human epithelial cells to examine whether a patient-friendly application of the LED-ETT is possible.

The total current of 25 mA DC at 16 V does not exceed the release threshold in the event of a fault, so there is no physical hazard from electric current [14]. With such a low current, it is possible to operate the system with two 9 V batteries connected in series, so that it can be used as a mobile system for a duration of up to one day. By using a different circuit layout with dedicated constant current sources, the total current could be further reduced to 3.125 mA per circuit, which would result in increased electrical safety.

Regarding the test setup, which deviate from the real application, the glass tube applied as a model trachea with 30 mm has a larger diameter than a real trachea [15] and has a smooth transparent surface. In addition, the human trachea is not full of secretions. However, they represent worse conditions compared to the real application due to the higher distance of the light source to the trachea and the filled glass tube. However, the real surface of the trachea and any folds and grooves that occur are not considered in the model.

5 Conclusion

This study demonstrated that irradiation on *A. kookii* at 450 nm is possible with an irradiance as low as 4.2 mW/cm² to reach a reduction of 1 log level within 24 h. No limitation of photoinactivation was observed for lower irradiances. Rather the inactivation effect related to the applied dose increased for this wavelength. This is a finding leading to the assumption that it might be possible to achieve prevention of bacterial colonization in an ETT while at the same time being as gentle as possible towards tracheal epithelium.

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