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Investigating the effect of low-level laser therapy on acne bacteria

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Abstract

Acne is one of the most common skin diseases today and leaves a bad influence on the psychology of the patient because of scars and permanent deformation on the skin. Low-level laser phototherapy methods have been proposed for research in the treatment of acne. This paper investigates the inhibitory ability of blue laser (532nm) at a power of 5mW lighting directly on petri dishes with C. acnes – causing acne vulgaris. Our team conduct the survey in 3 exposures 48 hours apart with 4 times intervals (5 minutes - 10 minutes - 20 minutes - 30 minutes) to find the most suitable course of treatment. With the results of the number of bacteria that obtained on the surface of the petri, exposes 3 irradiations for 30 minutes, it reduced by 82% compared to the sample. Therefore, the result indicate that this is the most effective period to achieve treatment for a course of 3 times irradiate 48-hour-period.

1 Introduction

Acne is a skin disease, caused by hormones that stimulate sebum secretion, abnormal keratinization of the hair follicles, an increase in the number of microorganisms on the skin, and an inflammatory reaction of the cells [1]. One of the microorganisms involved in the inflammatory

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response of acne is Cutibacterium acnes (C. acnes), a Gram-positive bacterium that changes the lipid composition of sebum, causing moderate inflammation.

Acne is more common in women than in men of all ages. The prevalence of adult acne is highest in people aged 20–29 years (50.9% of women and 42.5% of men). This percentage decreases with age, 15.3% of women and 7.3% of men are over 50 years old [2]. A 10-year study analyzing acne patients in a dermatology center reported that the incidence of post-adolescent acne cases was approximately 30% of all acne cases seen, in which female dominated (64.1-69.6%) during the 10-year study period [3].

Antibiotics, retinoids, and other substances such as benzoyl peroxide, salicylic acid, azelaic acid and alpha-hydroxy have been shown to be effective in the treatment of common acne [4]. However, these drugs are accompanied by side effects such as skin irritation, peeling, tanning [5] and prolonged treatment time leading to resistance to antibiotics and acne drugs. The effort in finding new treatments, light therapy in acne treatment was discovered, which is considered as a promising method of quick and effective treatment.

From the comments on the above situation, our group decided to choose the topic "Investigating the effect of low-level laser therapy on acne bacteria". The goal is to find the right time to develop into a course of acne treatment. In this paper, we investigate the effect of 532nm green laser on Cutibacterium acnes in vitro.

2 Methods and Materials

2.1 Materials

For the biological material, the bacteria strain Cutibacterium acnes (C. acnes) was obtained from the Biotechnology laboratory of the Department of Chemical Engineering, University of Technology (HCMC). The bacteria were cultured on Trypticase Soy Agar (TSA) for 48 hours and incubated at 37℃ in anaerobic medium. For the light source, we used DC 3V green laser diode with 532 nm wavelength, 5mW power and continuous laser emission.

2.2 Methods - Using In vitro Assay

The parent bacteria sample was gram stained for identification and confirmed for the correct species before isolating onto new agar plates.

We inoculated bacteria isolates in TSA medium during the first 48 hours of incubation at 37℃ under anaerobic conditions.

Experimental instruments were boiled and disinfected at a high level for 20 minutes and placed in a biological safety cabinet under UV light.

First exposure experiment: we took 10 µl of bacteria and diluted 10^{-5} times (dilution enough for investigation) in the ratio 1:9. Then, we inoculated 1µl of diluted solution onto 5 agar plates as control plates, 5 min (1), 10 min (1), 20 min (1), 30 min (1) dose as together. The agar plates were exposed to the laser to emit continuously according to the survey time. After laser exposure, the petri dishes were incubated at 37℃ in an anaerobic environment for 48 hours for the bacteria to form new colonies.

Second exposure experiment: we took 10 µl of bacteria from the plate for 5 minutes (1) survey and diluted 10^{-5} times (dilution enough for investigation) in the ratio 1:9. Then, we inoculated 1µl of diluted solution onto a new agar plate for 5 min (2). We repeated the dilution operation on each plate (10p, 20p, 30p) separately due to the difference affected by the laser in the 1st exposure. The agar plates were exposed to the laser to emit continuously according to the survey time. After laser

exposure, the petri dishes were incubated at 37℃ in an anaerobic environment for 48 hours for the bacteria to form new colonies.

Third projection experiment: The operation is repeated as in the 2nd projection.

The test was repeated several times to give the most satisfactory and reasonable results.

2.3 Statistical analysis

Colonies with high tissue, opaque yellow, about 0.5 - 4 mm in size depending on the time and culture medium [6].

We counted all the single colonies growing on the medium and selected plates with a colony count of about 30 – 300. The results were calculated (based on the number of colonies counted and the dilution to calculate the number of colonies in the original solution).

Quantitative results: results are returned in quantity + CFU/ml with quantitative cultures.

3 Design

To study the effect of low-power laser on C.acnes bacteria will go through three stages: inoculation, laser irradiation and culture. In order to be able to qualify for the three stages, two models from styrofoam boxes including Inoculation cabinets/ Biological safety cabinets and Bacterial culture - laser irradiation cabinet were developed.

3.1 Inoculation cabinet/ Biological safety cabinet

The inoculation stage requires that the culture environment be disinfected, and the laboratory equipment must be sterilized. Therefore, to limit the risk of contamination from the environment, the model of incubator / biological safety cabinet has been designed and manufactured.

Figure 1: Culture cabinet

The incubator is lined with foil inside, making it easy to clean before and after inoculation. The mica layer both acts as a shield and helps to observe the inside of the incubator, thereby limiting external agents and making it easier for experimenters to manipulate. In addition, the cabinet is also equipped with led lights, UV lights, alcohol lights and exhaust fan.

3.2 Preparation steps before and after the experiment

First, the instruments will be sterilized (boil with a high level of sterilization within 20 minutes of boiling) and moved through an incubator immediately after sterilized. While waiting for boiling, wipe the inside by alcohol and turn on the UV light to kill bacteria in the incubator.

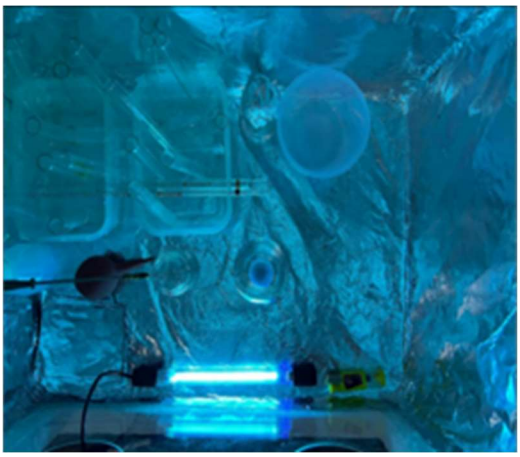

Figure 2: UV light turns on to disinfect inside the box

Next, the process of extracting distilled water and inoculating bacteria will be carried out next to the alcohol lamp to ensure that the practice area is not contaminated.

Due to the long incubation time, the alcohol lamp is always burning, causing the high temperature inside the cabinet that affect the operators and the work progress. That's why the team decided to install a fan on the lid of the cabinet so that hot air could be brought out from the inside. Although air from the outside can enter through the hole in the mica plate (the hole is cut for the experimenters to put their hands inside) will create a moving gas flow in the cabinet. However, the alcohol lamp is always lit, so foreign bacteria can be prevented from entering the sample.

After the experiment, the instruments and the incubator will be cleaned and disinfected.

3.3 Bacterial culture - laser irradiation cabinet

Bacterial culture and laser irradiation were carried out in the same styrofoam box divided into 2 chambers by a layer of foam and mica. The box is lined with foil inside, which both retains heat on the side of the culture chamber and helps to limit laser scattering on the side of the laser chamber.

Figure 3: Bacterial culture cabinet and laser irradiation

Preparation steps:

1. The cabinet's lid is opened and interior surface disinfected

2. UV lamp is installed in the laser chamber

3. The cabinet is sealed with candle glue and duct tape. Then the UV lamp is turned on to kill bacteria for 10 minutes

Bacterial culture chamber: C. acnes bacteria requires two conditions to grow well: temperature 37℃ and anaerobic environment. To maintain a temperature of 37℃, we use a filament lamp to heat up and it will automatically turn on if the temperature is below 36.5℃ and will turn off if the temperature reaches 37 ℃ (the highest temperature achieved by the cabinet is 38 ℃). via a commercially available temperature sensor circuit (Egg incubator circuit). The temperature displayed by the circuit is checked by an electronic temperature measuring device.

To ensure anaerobic conditions, the cabinet is sealed with candle glue, silicon and isolated from the outside, the partition is also checked and reinforced for clefts regularly. After being laser irradiated, the petri dish containing bacteria will be covered with food wrap, and the dish has a time period for identification on the lid.

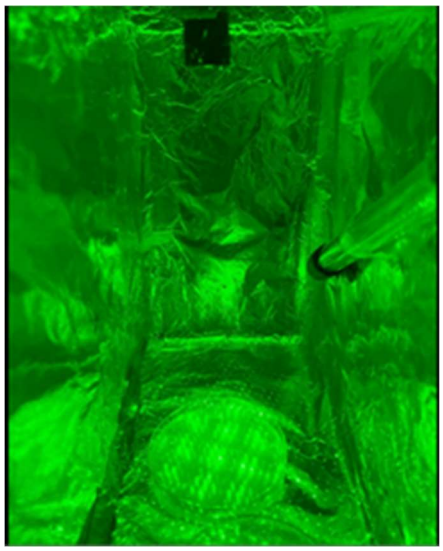

Figure 4: The petri dish has been opened and the light is in progress

Laser irradiation chamber: The bacteria after being inoculated into the petri dish will be brought into the projection cabinet. Next, the team proceeded to open the lid of the petri dish by the glove to avoid contaminating the plate with foreign bacteria.

After being laser irradiated for the specified time, close the lid of the petri dish and take it out to the outside and wrap the food wrap around the dish. Then, put it into the bacterial culture chamber.

Because of many factors, both models cannot meet the requirements of the laboratory, but can only minimize the infection during the experiment.

4 Result and discussion

Times	sample (CFU/ml)	5 minutes (CFU/ml)	10 minutes (CFU/ml)	20 minutes (CFU/ml)	30 minutes (CFU/ml)
1	2.1×10^{10}	1.9×10^{10}	1.6×10^{10}	1.4×10^{10}	1.1×10^{10}
2	2.1×10^{10}	1.4×10^{10}	1.1×10^{10}	10^{10}	8.5×10^{9}
3	2.1×10^{10}	9.7×10^{9}	8.2×10^{9}	5.7×10^{9}	3.6×10^{9}

Table 1: Number of bacteria after 3 doses of irradiation

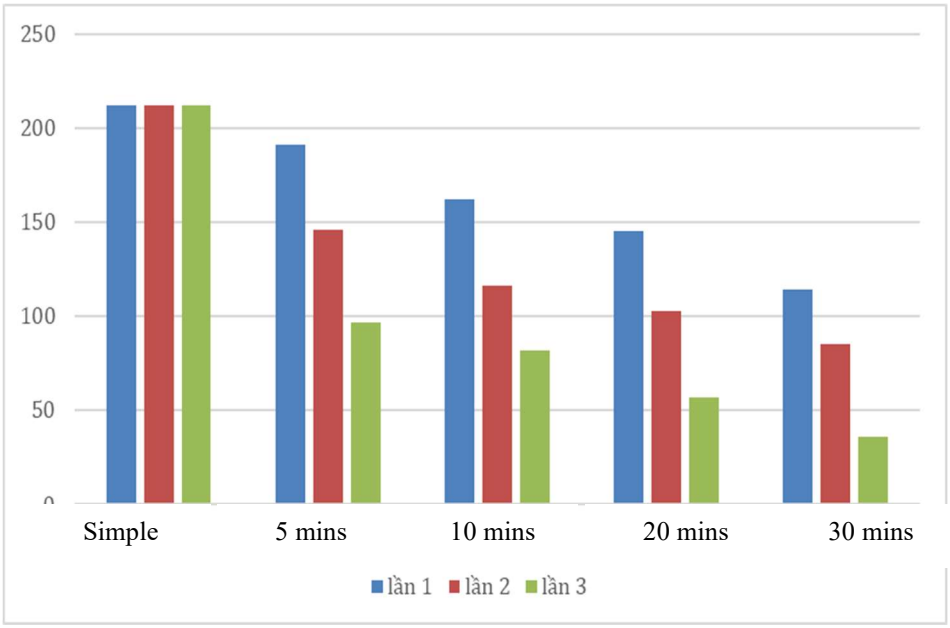

Figure 5: The number of bacteria on the surface of petri

After three exposures with 4 corresponding time periods, the team obtained results on the influence of 532nm wavelength blue laser radiation on petri dishes containing bacteria as follows:

First time the number of CFUs reduced after 5 minutes of irradiation was 9.5%, after 10 minutes - 23.8%, after 20 minutes - over 33%, after 30 minutes - 47%.

Second time: the number of CFUs decreased after minutes of exposure was over 33%, after 10 minutes - 47.6%, after 20 minutes - over 52% and after 32 minutes, the numbers of colonies decreased by 59%.

Third time the number of CFUs after 5 minutes under the effect of green laser radiation was reduced by 53.8%, after 10 minutes - over 60%, after 20 minutes - over 72%, after 30 minutes - over 82%.

The number of colonies on the petri dish was reduced by more than 82% compared to the control sample after irradiating 30 minutes with 3 doses that are 48 hours apart, this is considered the most effective result in the period of survey time. The irradiation time changed from 5 minutes to 30 minutes, which reduced the survival rate of C.acnes bacteria from 50 to over 80% compared to the control sample.

Figure 6: The difference between original plate and 30 min plate after 3 doses of irradiation

5 Conclusions

This topic has achieved the goal of studying the effect of laser using 3V DC green laser diode with wavelength 532 nm, power 5mW, continuous emission at specified time on C. acnes bacteria samples.

The team has successfully designed and manufactured a simple bacterial culture - bio-safety cabinet - laser irradiation model for 5 petri dishes including a Comparison dish and 4 projection plates with different time: 5 minutes, 10 minutes, 20 minutes, and 30 minutes.

However, the results of the above model still have many errors due to the influence of objective factors such as anaerobic conditions in the incubator, aseptic conditions in the incubator and laser cabinet.

We fully adhere to the experiment and publication guidelines of Ho Chi Minh City University of Technology and Vietnam National University Ho Chi Minh City.

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