

# Using invasive and non-invasive methods to evaluate the therapeutic effect of dielectric barrier discharge (DBD) Plasma in skin graft surgery

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## ABSTRACT

Skin graft is effective method to reconstruct large lesions on the skin. Long-term injuries such as burns, skin ulcers, skin flaps, skin grafts... can affect the health of the patient. Skin plays a very important role because it protects the body from dehydration, helps regulate temperature, helps prevent pathogenic bacteria or viruses from entering the body and minimizes skin deformation. In recent years the cold plasma has been used to aid the treatment of chronic skin wounds, burns, ulcers,... To assess the effectiveness of the wound healing process of Plasma, this study uses non – invasive (assess the shrinkage of the wound, the temperature change of the wound) and invasive methods (regeneration of skin tissue structure) to evaluate the therapeutic effects of Dielectric Barrier Dissections Plasma (DBD Plasma) in skin graft surgery. Experimenting with the entire skin graft model on mice, this use of DBD Plasma treatment along with clinical evaluation methods, this is to compare wound healing time between wounds treated with plasma DBD and untreated wound. After 21 days of experimentation, evaluation methods and results processing tools, it was shown that wound healing of skin lesions on mice of the wound area treated with DBD Plasma faster than nontreatment about: (1) The surface shrinkage rate is about 25%; (2) The average wound temperature is less than 0.5- 1°C; (3) Healing time is faster from 5 to 6 days. Therefore, DBD Plasma is able to be a potential treatment for wound healing combined with skin graft surgery in the future. The initial study of DBD model is the premise from which could be broader in other animals such as rabbits and pigs with skin structure closer to the human skin. And further, the DBD Plasma shows the potential for preclinical application to human skin graft.

**Key words:** DBD plasma, skin graft surgery, healing wound

## INTRODUCTION

Currently, more and more researches applying DBD Plasma in the field of biomedical. DBD Plasma device creates free radicals, reactive species,...<sup>1,2</sup>. These free radicals, reactive species will react to the outside environment and inside the wound including bactericidal<sup>3</sup>; stimulates and promotes cellular functions such as leukocyte and fibroblast attraction leading to faster inflammation as well as stimulating blood vessels to help the recovery process occur faster<sup>4,5</sup>. From the research results, a range of benefits of DBD Plasma device such as room temperature range, no direct contact, portable, high treatment efficiency, ...<sup>6</sup>.

However, the application of DBD Plasma in skin grafts surgery is still limited because there are not many experimental models proving the effectiveness of DBD Plasma on skin grafts surgery. Therefore, the objective of this study is to use methods of assessing wound contraction, changes in wound area temperature, and

regenerating skin tissue structure, in order to evaluate the effectiveness of DBD Plasma treatment in skin graft surgery. Aiming to open a new application direction for DBD Plasma equipment. Based on the goals that need to be achieved, the research sets out the following tasks and develops the following implementation processes:

- Understand the principle of operation and the basic studies of the interaction between the cold plasma with tissues and the effect of the physical components contained in the Plasma on living tissues.
- Experimental design on mice.
- Use the method of evaluation of shrinkage, temperature assessment, skin tissue structure assessment to evaluate the effectiveness of DBD Plasma's wound healing process.

## MATERIAL AND METHODS

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### Object of study

Male lab mice race BALB/C (Figure 1). The study choose mice by following reasons:

They have the same skin structure as human. Life cycle is short, giving birth many babies, having a huge number which means that it is suitable for doing research and experiment. It is easy to breed, cheap price, do not have any conditions about cages and food. They have fast transformation so people find it easy to collect data during the experiment.

Source: INSTITUTE OF DRUG QUALITY CONTROL HO CHI MINH CITY

Average weight: 25g – 27 g



Figure 1: Mice used in experiments

### Devices and Chemical substance

Anesthetic with the rate of ketamin:xylazine:water is 3:3:4; Formalin 10%; Hair removal cream; Rubbing alcohol (Figure 2).

Thermal camera FLIR Lepton: showing the temperature of the wound (Figures 1 and 3) and DBD plasma for the skin graft treatment (Figure 4).

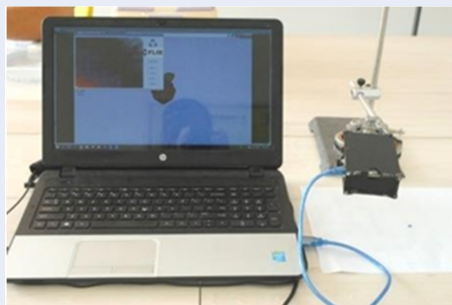


Figure 3: Thermal camera FLIR Lepton

Plasma devices include remote control, LCD screen, plug of power connector, control button, status LCD light, Micro USB charging port, wire, touch array.



Figure 4: Pasma devices

Operation principle: DBD plasma is created when putting a AC power source into two electrodes which are spaced by a material layer, this material layer prevents two electrodes from discharging.

### Experiment

Mice anaesthetic process: anesthetic include ketamine, xylazine and distilled water mixed with ratio: ketamine: xylazine: distilled water = 3:3:4. Anesthetic dose has been injected per time is 0.11-0.12  $\mu$ L, anesthetic time is about 30 to 40 minutes. Beside that, make sure that mices do not die during experiment time, compliance with animal protection laws. After shaping the size (1.5x1 cm) of the skin graft, use a scalpel to cut the skin along the line to make two similar wounds. Only incise to the dermis, avoiding incision into the subcutaneous muscle. The left hand holds the surgical clamp, the edge of the skin has been incised and slightly raised. The right hand holds the surgical scissors that cuts with the tissue under the skin (or the subcutaneous fat layer). Using tongs, separate the skin piece from the back of the mouse, placing it face up on a clean, dry tray. Use a paper towel to wipe the blood around the cut area. Then put the skin piece back in position, adjusting the edges of the skin closely. Then use a surgical needle to sew the wound (Figure 5).

Healing process is compared and evaluated that by making the same skin graft on one mice's back. NT: without treatment; P: with plasma treatment.

Treatment process happened in on first five days (two days: antibacterian/anti-inflammatory; three days: epitheliogenetic period).

Durring this process, skin graft areas were taken photo by Nikon D3000 Camera, Thermal Camera.

Results was monitored and collected everyday. The skin graft areas were cut as sample and undergo HE staining at first day, seventh day and fourteenth day.

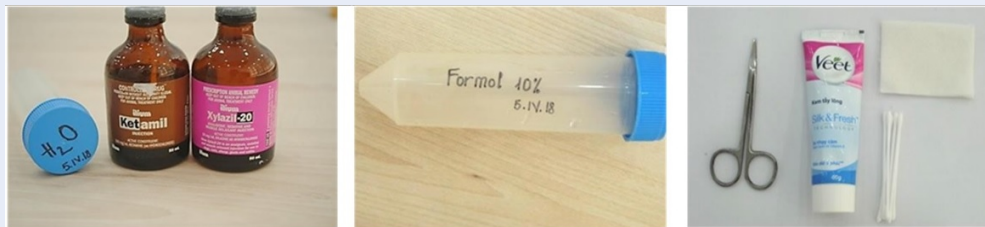


Figure 2: Chemicals used in the experiment

Since the sixth day, two skin graft areas did not receive any treatment and continuously being monitored and evaluated by using Image J software to measure wound area of two wounds and then using Matlab and excel software to analyze and compare.

## RESULTS AND DISCUSSION

### Normal images results

Figure 6 and Figure 7 showed the results of wound open surface following the time:

Day 1: The newly created wounds in the size of the wounds are uniform with each other, the color of the grafted skin and the surrounding back area remain similar, no change.

Day 7: There was a big difference in color and elasticity between wounds. In untreated wounds, scales are formed that cover the entire wound area, the skin turns dark brown, hard, without elasticity, and no hair grows from this area. For wounds treated with DBD Plasma, scabs were formed only at the edge of the skin graft. In the DBD plasma treated wound, the skin still has elasticity, but it is still harder than the normal skin, the skin color in this area only turns a bit yellow, not dark brown like untreated skin marks. And especially in wounds treated with DBD Plasma, the hair is still growing normally.

For all signs above such as scabs were formed only at the edge of the skin graft, skin still has elasticity, the hair in the skin is still growing normally, show that the skin graft has been received and not eliminated. Although both wounds have partially shrunk, there is still no big difference, between the two wounds only about 1.5% difference. By using Image J software to measure wound area of two wounds and then using matlab and excel software to analyze and compare.

Day 14: By this time, it is already possible to see a big difference between non-treatment wound and DBD plasma treatment wound in terms of area, color and elasticity. Compared to the untreated wound, the wound treated with Plasma DBD shrunk more, the area of the two wounds was about 17-20%. The skin

color of the treated area is brighter, only slightly yellowish brown, not black as the untreated stain. There is still hair on the skin of the treated area, while the untreated spot is not.

### Thermal results

From the results of temperature assessment of the wound site in Figure 8, it can be seen that the wound treated with DBD Plasma always has a lower temperature than the wound without treatment about 0.5 - 1°C. And wounds treated with Plasma DBD always have temperatures closer to normal skin temperatures than untreated wounds.

### HE staining results: comparison and evaluation of area, wound healing time.

From HE results in Figure 9, a difference in the structure of skin tissue could be seen in both wounds. In untreated wounds, there is a stratum corneum, thick cuticles, and no follicles. In the wound treated with Plasma DBD, there is no horny layer, thinner epidermis with many hair follicles.

This is the stage of the proliferation process. Thus, it can be seen that the proliferation process in wounds treated with DBD Plasma takes place faster than the untreated wound.

## CONCLUSION

By non-invasive methods (assessment of wound contraction, changes in the temperature of the wound area) and invasion (regeneration of skin tissue structure), the topic has confirmed the effectiveness of DBD Plasma in promoting the healing process due to skin grafts.

Wounds treated with DBD Plasma still have normal hair growth, color, skin elasticity that does not change much compared to normal skin, the rate of keratinosis takes place later but scales earlier with untreated wounds. In wounds treated with DBD Plasma, the wound is larger than the untreated wound for about 6 days.

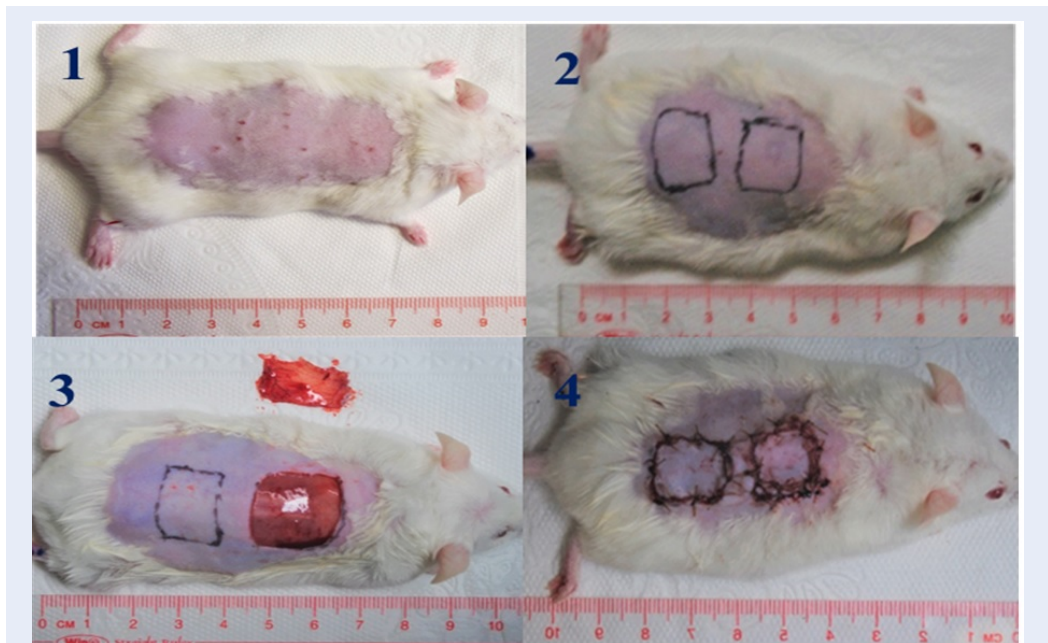


Figure 5: Making the skin graft in mice.

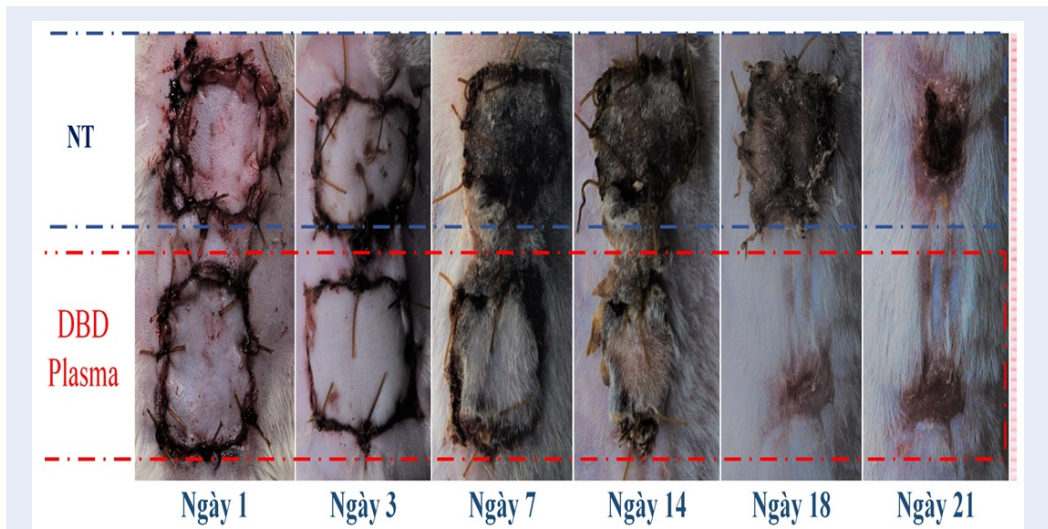


Figure 6: Monitor the shrink of wound area through normal images measured by the time (NT: non-treatment; DBD Plasma treatment)

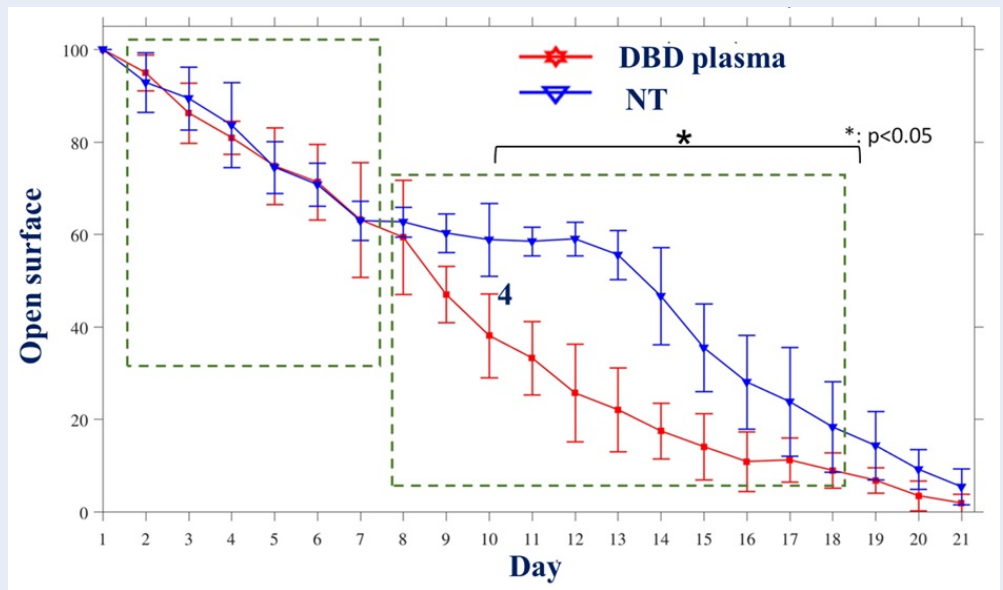


Figure 7: The open surface measured by the time

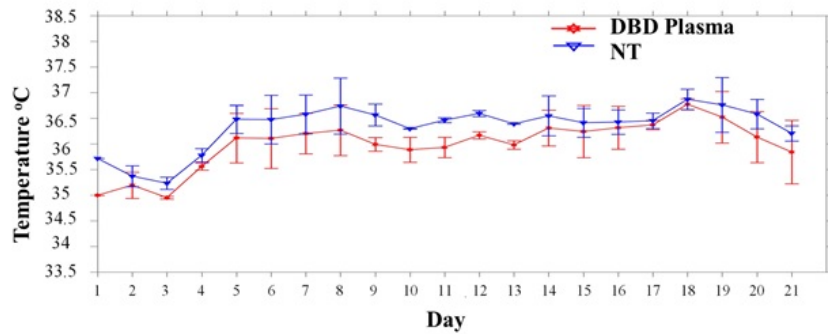
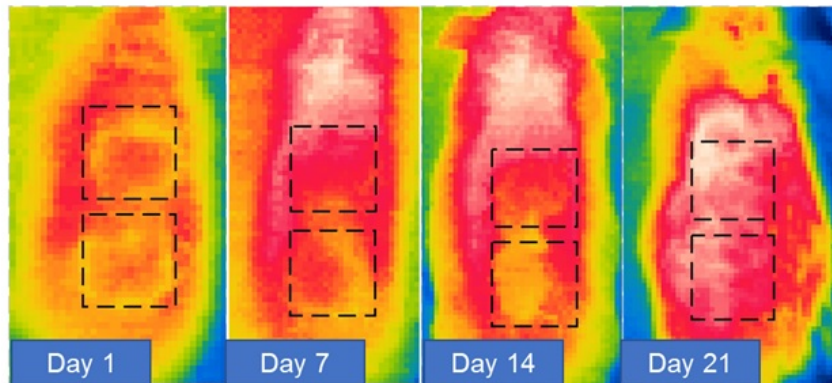
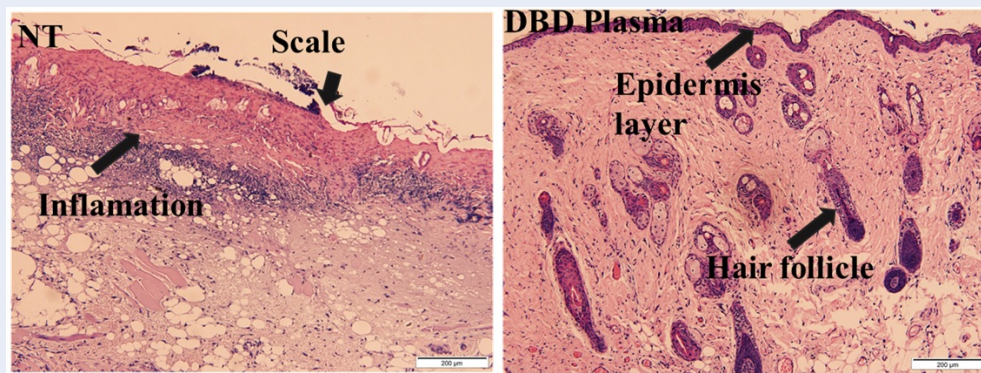


Figure 8: Thermal imaging



**Figure 9:** Images results taken by microscope at the seventh day of the NT and DBD plasma treated area

The temperature of the wound treated with DBD Plasma is lower than 0.5 - 1°C and is maintained closer to the normal skin temperature than the untreated wound.

In wounds treated with Plasma DBD, the structure of skin tissue recovers much faster than the untreated wound about 25%.

Thus wounds treated with DBD Plasma have a faster healing rate than wounds left untreated from 5 to 6 days. Plasma DBD device has a positive effect in speeding up the process of wound healing in skin graft surgery.

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### AUTHORS' CONTRIBUTION

All authors contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

### CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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