Enhancement of Ca^{2+} influx through transient receptor potential channels by flexible plasma patch therapy: psoriasis curing

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Abstract: A cold atmospheric plasma (CAP) patch is fabricated to cure skin diseases. This patch has electrodes that induce surface dielectric barrier discharge on a flexible polymer film surface. The effect of the CAP patch on the opening of transient receptor potential channel is evaluated for psoriasis curing. The CAP patch induces the opening of calcium channels in keratinocytes, thereby restoring abnormal keratinocyte differentiation and the collapse of the tight junction; thus, alleviating psoriatic symptoms. The findings indicate that the proposed portable CAP patch can help treat inflammatory skin disorders, especially psoriasis.

Keywords: plasma patch, skin disease, ROS.

1. Introduction

Plasma technology has been proved to be promising in various fields such as chemistry, biology, physics, and biotechnological and medical science.[1] Recently, the potential for skin treatment via dielectric barrier discharge plasma has also been demonstrated.[2,3] Plasma can be generated artificially by heating a gas or applying electromagnetic fields. In this manner, cold atmospheric plasma (CAP), partially ionized gas at atmospheric pressure and room temperature, is capable of generating reactive oxygen species (ROS), reactive nitrogen species (RNS), electric fields, ions, electrons, and ultraviolet (UV) and visible rays.[4–9]

We fabricated a flexible CAP patch consisting of a portable power unit to overcome these issues. The flexible CAP patch is a polymer film with printed metal electrodes. The CAP patch uses surface dielectric barrier discharge to generate ROS and RNS on flexible polymer film surfaces. The printed metal electrodes induce an electric field of 10–30 kV cm^{-1}. A patient will remain safe during the CAP operation despite the strong electric field because the metal electrodes in contact with the skin are electrically grounded while applying a high voltage to the insulated electrode. We chose psoriasis to assess the clinical application of the CAP patch among all inflammatory skin diseases.

2. Results and Discussion

The CAP patch device consists of a CAP patch and a portable power unit (PPU). The CAP patch is a combination of a high voltage insulating film, a dielectric barrier polymer film, a high voltage electrode, and a ground mesh electrode. The CAP patch uses a 120 μm thick PET film as a flexible dielectric barrier, allowing the patch to be adhered to curved skin surfaces. The mesh electrode is screen-printed on the film, so the area of the CAP patch can be easily expanded to 100 cm^2. The PPU consists of a main control unit, a high voltage power supply and a lithium ion battery. The PPU supplied a sinusoidal high voltage (maximum voltage: 2–2.5 kV). CAP patches use ambient air to create surface plasma. Air plasma is generated at the edge of the hexagonal electrode. Figure 1 shows air plasma on a CAP patch. When the PPU supplies a 2 kV sinusoidal voltage, the field strength at the edges of the mesh electrodes is above 30 kV cm, which is sufficient to induce an air plasma on the patch surface. We used round CAP patches with a diameter of 1.5 cm in vitro experiments to cover well plates.

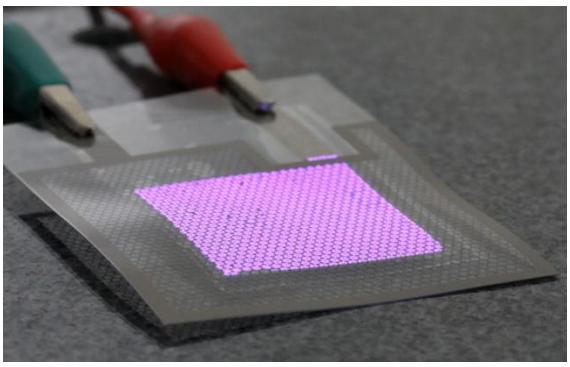

Figure 1. CAP patch

Characteristics of the CAP patches, such as power consumption, leakage current, surface temperature, and light emission, were measured using a diagnostic system. A CAP patch was attached to the wall of the diagnostic system. The wall contains a current probe, an optical lens with cosine collector, and a gas sampling port. Power loss was measured on the PPU signal. Plasma patches supply ozone to the skin. Therefore, the time-weighted average (TWA) of working hours per day must comply with Korea's 'Chemical Substances and Physical Factors Exposure Standard'. TWA means exposure time based on 8 hours per day. According to the recently issued domestic plasma generating medical device approval review guidelines, the TWA of plasma skin treatment devices is limited to 0.05ppm or less. The daily usage time is 10 minutes (1/6 hour), and the TWA was calculated by applying the ozone concentration according to the operation frequency. Under the condition of 60Hz frequency, TWA satisfies the specified value of 0.05ppm. However, it exceeds 0.05 ppm at 1000 Hz. Therefore, the CAP patch was operated at 60 Hz for in vivo and in vitro tests, as frequent discharge repetitions can cause thermal, optical, and electrical damage. Power dissipation at a

frequency of 60 Hz ranged from 0.7–2.2 mW cm^{-2}, where power dissipation did not increase the surface temperature from the initial temperature (18 °C). Optical emission and leakage currents were not detectable by the diagnostic system. Hexagonal pattern geometries such as line width and length were optimized to enhance plasma generation. The optimized geometry of the mesh electrode had a line length of 0.75 mm and a line width of 0.2 mm. This pattern has been applied to in vivo and in vitro tests. The concentrations of ROS and RNS supplied by the CAP patch were measured with ozone and NO_x detectors. At 2.8 kV discharge, the O_3 concentration increased rapidly to 400 ppb and became more saturated. The NO concentration was similar to the initial gas concentration. Nitrogen dioxide concentrations continued to increase to 38 ppb, below the regulatory limit for a 1-hour exposure (100 ppb). In practical use, ROS and RNS species are difficult to breathe through the respiratory tract due to their distance from the nose.

Calcium ions are important in regulating many skin functions, such as keratinocyte differentiation and skin barrier formation.[10] Abnormal differentiation and tight junction instability are observed in psoriatic keratinocytes due to lack of calcium influx. [9, 11] considering the importance of calcium in keratinocytes, we checked whether the CAP patch could induce calcium influx into cells. A fluorescence assay was performed using a calcium indicator (Fluo-3/AM) to evaluate calcium influx. Confocal microscopy and fluorescence measurements revealed increased intracellular calcium levels after CAP patch treatment in keratinocytes. We also performed patch clamp to identify calcium channels triggering calcium influx. In calcium channels, various transient receptor potential (TRP) channels participate in the formation and maintenance of the skin barrier, skin immunity, and inflammatory processes to establish skin homeostasis and contribute to many types of skin disorders.[11, 12] TRP channels have sensory It is expressed not only in nerve terminals, but also in many non-neuronal cell populations, including keratinocytes and skin-resident immune cells.[19] In particular, TRP subfamily V member 1, TRP subfamily A member 1, and TRPV4 have been shown to regulate psoriasis. [13–16] Therefore, we set out to investigate the electrophysiological response to AITC, an agonist of the whole cell A voltage-clamp analysis was used. 200 μM for TRPA1), capsaicin (1 μM for TRPV1) and GSK1016790A (1 μM for TRPV4), similarly sized control and CAP patch treated keratinocytes. Current amplitudes induced by each agonist for TRPA1, TRPV1 and TRPV4 were significantly increased by CAP patch treatment for 10 min, without any specific change in cell morphology, compared to the control group for TRPA1, TRPV1 and TRPV4. This indicates that the CAP patch induces the opening of calcium channels (TRPA1, TRPV1 and TRPV4), increasing calcium levels in keratinocytes.

3. References

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