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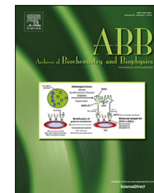

# Galectin expression in healing wounded skin treated with low-temperature plasma: Comparison with treatment by electronical coagulation

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ABSTRACT

Low-temperature plasma is useful for the care of wounded skin. It accelerates wound healing. However, the mechanism of this effect has not been fully elucidated yet. Galectin-1 is reported to accelerate wound healing via the Smad signaling pathway. In the present study to clarify whether or not galectins were expressed during the process of wound healing in the plasma-treated skin, we examined the effect of low-temperature plasma on galectin expression in the healing skin. We compared the effects of low-temperature plasma on the expression of galectin-1, -2, and -3 in the healing skin with those of electrocoagulation conducted with a high-frequency electrical coagulator. Immediately after the start of low-temperature plasma treatment following the incision made in the skin, a membrane-like structure was formed on the surface of the wound. Immunoelectron microscopy showed that these galectins were localized in the membrane-like structure of the plasma-treated skin. The expressions of these galectins were increased by the low-temperature plasma treatment, whereas they were inhibited by the electrocoagulation. These results suggest that galectins were involved in the wound healing of low-temperature plasma-treated skin. Galectins will thus be good markers for further examination of the effects of low-temperature plasma on the healing of wounded skin.

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## 1. Introduction

It is reported that low-temperature plasma is effective for hemostasis and promoting the healing of skin wounds and the regeneration of epithelial tissue [1–3]. The application of low-temperature plasma is also useful for the treatment of chronic inflammatory skin diseases [4,5].

The equipment that we developed to supply plasma to form blood clots can keep the temperature below 40 °C and thus avoid thermal injuries commonly observed after high-frequency electrocoagulation treatment (HFEC). The fibrous membrane-like structure was formed by this equipment [2,6]. In this present study we examined the components of this fibrous membrane-like structure. We performed immunoelectron microscopy of the membrane-like structure induced by the plasma treatment to study the mechanisms underlying clot formation.

Galectins are a family of animal lectins having both galactose-binding ability and amino-acid sequences characteristic of galectins [7–16]. Galectins are localized in many tissues and play many important roles, being involved in cell adhesion, differentiation, transcription, and wound healing [17–26]. Recently, it was reported that galectin-1 accelerates wound healing by regulating the

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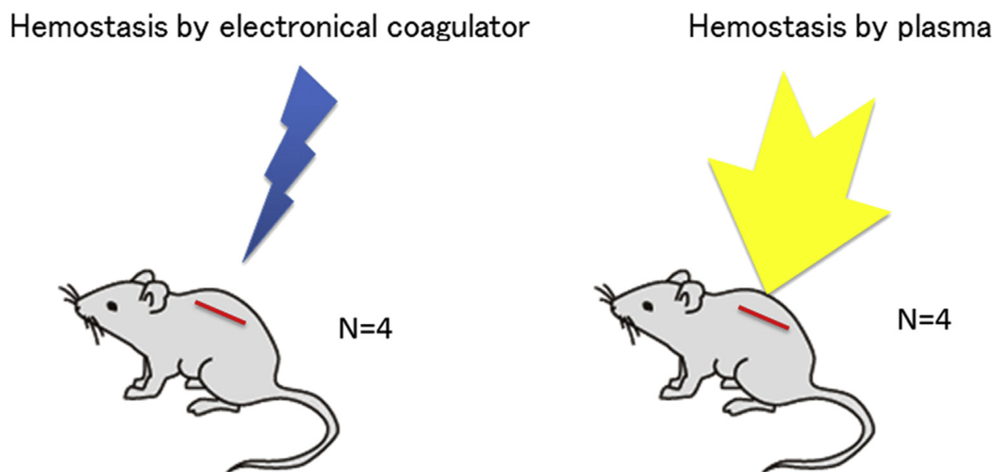


**Fig. 1.** Scheme for the treatment with plasma (right) or high-frequency electronical coagulator (left) for bleeding from the wounded skin. N = 4.

neuropilin-1/Smad3/NOX4 pathway and ROS production in myofibroblasts [27]. This galectin also plays an important role as an inducer of extracellular matrix formation and TGF-β-independent conversion of fibroblasts into myofibroblasts [22]. In this study we compared galectin expression during the healing of skin wounds treated with low-temperature plasma with that obtained with electrocoagulation. Furthermore, by using microarray and real-time PCR techniques, we also examined what kinds of genes were up-regulated or down-regulated in their expression in healing wounded skin treated with electrocoagulation versus their expression in that treated with low-temperature plasma.

## 2. Materials and methods

### 2.1. Animals and study approval for animal experiments

Female C57BL/6 (B6) mice were obtained at 8–12 weeks of age from Charles River Japan Inc. (Yokohama, Japan) and were housed 4 animals per cage pre-surgery and alone post-surgery under standard housing conditions. All animal experiments were performed by using an experimental protocol approved by the Ethics Review Committee for Animal Care and Use Committee of the *National Institute of Advanced Industrial Science and Technology (AIST: animal 2014-022 C).*

### 2.2. Treatment with low-temperature plasma at normal atmospheric pressure

For the plasma treatment, an instrument developed by AIST was used [2,28–30]. The instrument produces plasma by using a dielectric barrier discharge (66 kHz, sinusoidal peak to-peak voltage of 6.0 kV applied to an electrode) [2,28,29].

For plasma discharge, pure helium with a flow rate of 2 standard liters per minute was supplied, and the plasma flared from the nozzle of the instrument's handpiece. The target material was placed at a distance of 15 mm. The plasma flare from the instrument had an effective electrical current of 0.37 mA collected at the target position [31,32].

### 2.3. Surgical procedure

The dorsal surface of the mice was shaved with an electric clipper and sterilely prepped with 70% alcohol gauze sponges. After the animals had been anesthetized with isoflurane, 2 lateral full-thickness wounds per mouse were created with a scalpel and Westcott scissors, 40 mm cranial from the tail head and 10 mm lateral from the spine (Fig. 1).

Generated wounds were treated by either plasma technology or with the high-frequency electronical coagulator (HFEC). The details of the conditions of the equipment to generate plasma for bleeding control were described in our previous studies [2,28]. Briefly, the peak-to-peak voltage, $V_{p-hp}$, applied to the electrode was within the range of 6–10 kV, and the frequency range of the sinusoidal wave was between 10 and 70 kHz.

### 2.4. Histology of the skin

Mice were sacrificed at days 0, 3, 5, and 7 days after the treatment, at 1 h before which BrdU (Sigma Co., St. Louis, MO) was intraperitoneally injected at a concentration of 75 mg/kg body weight. Skin tissue including the wounds were excised with a scalpel (Surgical Blade No. 11, Feather Safety Razor Co., Ltd. Osaka, Japan), and fixed in neutral buffered 10% formalin overnight for routine processing for embedment in paraffin.

For BrdU staining, the sections were treated with 4 N HCl for 20 min at 37 °C, neutralized with 0.2 M boric acid-borate buffer at pH7.6, and washed with PBS. Mouse monoclonal antibody against BrdU (dilution 1:1,000, clone BU-33, Sigma Co.) was reacted according to the protocols suggested by the supplier. The immune complex was visualized by the ABC method using a Vector stain ABC kit (Vector Laboratories Inc., Burlingame, CA).

### 2.5. Electron microscopic analysis

For transmission electron microscopy (TEM), skin was fixed for 24 h in phosphate-buffered 2.5% glutaraldehyde (Wako). Post-fixation was performed for 1 h in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) in an ice bath. The specimens were embedded in Epon 812. Ultrathin sections were cut and stained with uranyl acetate and lead citrate and examined by TEM (JEM-1011, JEOL) [33].

### 2.6. Immunohistochemistry of galectins in the skin

Immunostaining was carried out as described previously [7]. Skin was fixed overnight in 10% formalin. Frozen sections (4-μm thickness) were cut, and antigen retrieval was carried out at 100 °C for 10 min. Sections were treated for 30 min with 1.5% $H_2O_2$ in PBS

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