

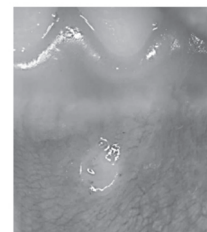
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研究題目：The effect of CO<sub>2</sub> laser on promoting wound healing of ulcer in mice oral mucosa

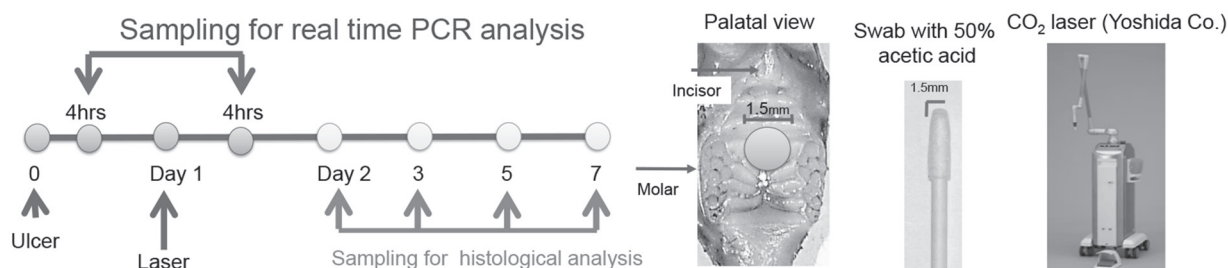
## BACKGROUND

Aphthous stomatitis is the painful recurrent oral ulceration with disturbing patients' daily activities such as eating, swallowing and speaking. However, there has been no curative therapy for it. The carbon dioxide (CO<sub>2</sub>) laser has been reported to help wound healing, and become a useful tool for pain relief of ulceration or stomatitis in clinical situation. However, the healing mechanisms by CO<sub>2</sub> laser treatment have not been completely understood.

Aphthous stomatitis



## METHODOROLOGY



### • Ulcer induction in mice

To create the ulcer, a swab with 50% acetic acid was put on the mice palatal mucosa for one minute under the general anesthesia.

### • Irradiation by CO<sub>2</sub> laser

Next day of ulcer was induced, The level of Low- (1.0w, pulse frequency 1ms) or High-CO<sub>2</sub> laser (3.0w, pulse frequency 2ms) were performed on the ulcer and surrounding tissue for 10s.

### • HE staining and immunostaining

After dissecting, palate tissue was fixated with 10% formalin.

#### 【 First antibody 】

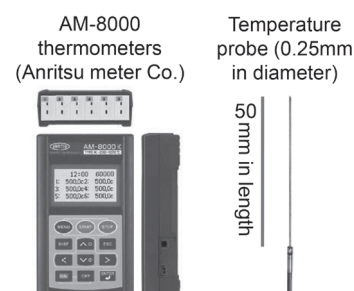
Monoclonal Rat Anti-Mouse Ki67 (DAKO Japan)

Polyclonal Rabbit Anti-Mouse Heat Shock Protein 70 (Cell signaling. USA)

Monoclonal Rat Anti-Tenascin C (R&D SYSTEMS. USA)

### • Temperature measurement

Thermometers were used to measure the temperature change caused by low- or high- CO<sub>2</sub>



laser treatment in palatal surface and inside of epithelium separately.

- **Real-time PCR**

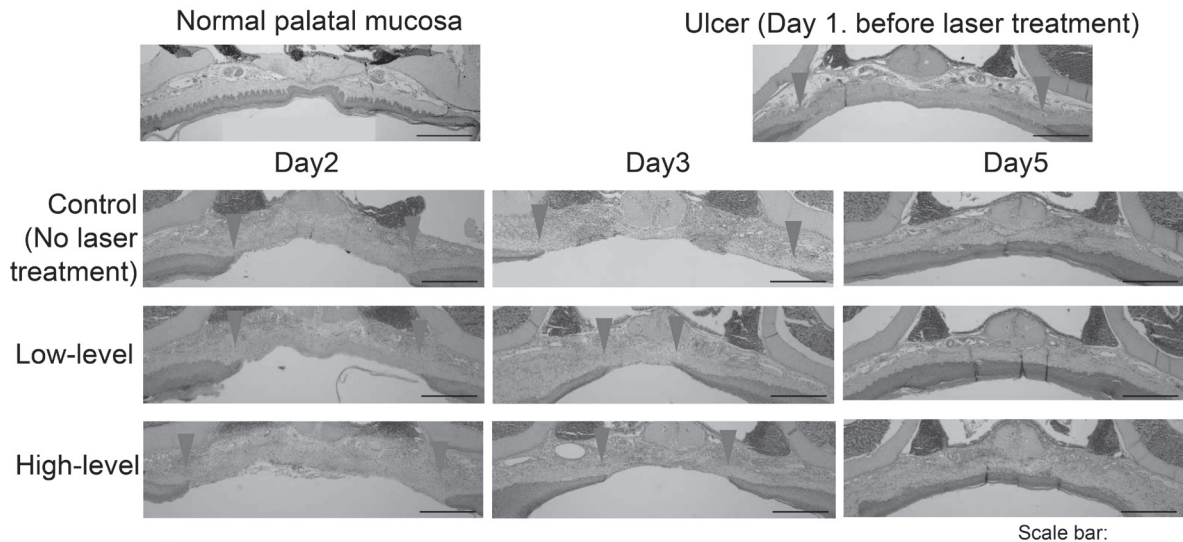
Ulcer lesions and surrounding tissues were collected separately 4 hours after laser irradiation and then homogenized in TRIzol reagent (Thermo Fisher). Total RNA was extracted as per the manufacturer’s recommendations. The mRNA expression of Heat shock protein 70 (HSP70), Tenascin C (TnC), were examined using SYBR system. GAPDH was used for normalization.

- Mann-Whitney U test was used for statistic analysis.

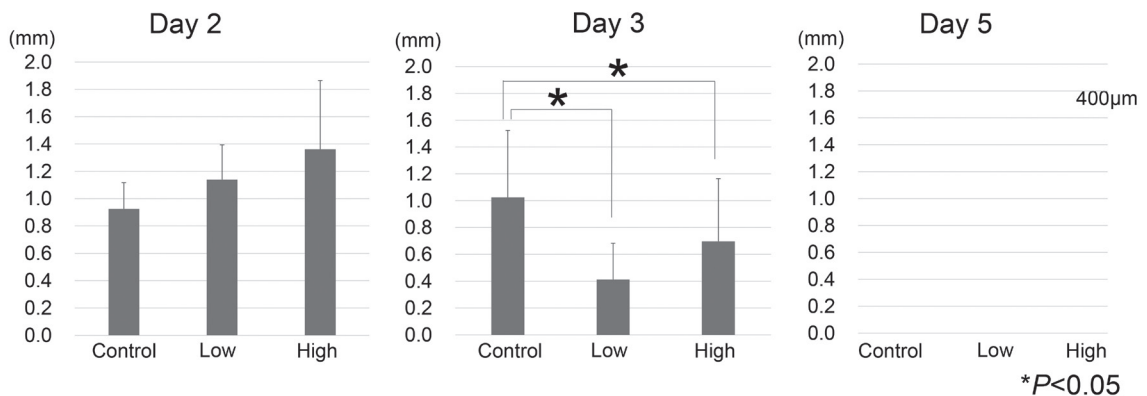
## RESULTS

### 1. Healing process of ulcer with/without CO<sub>2</sub> laser treatment

#### H & E staining



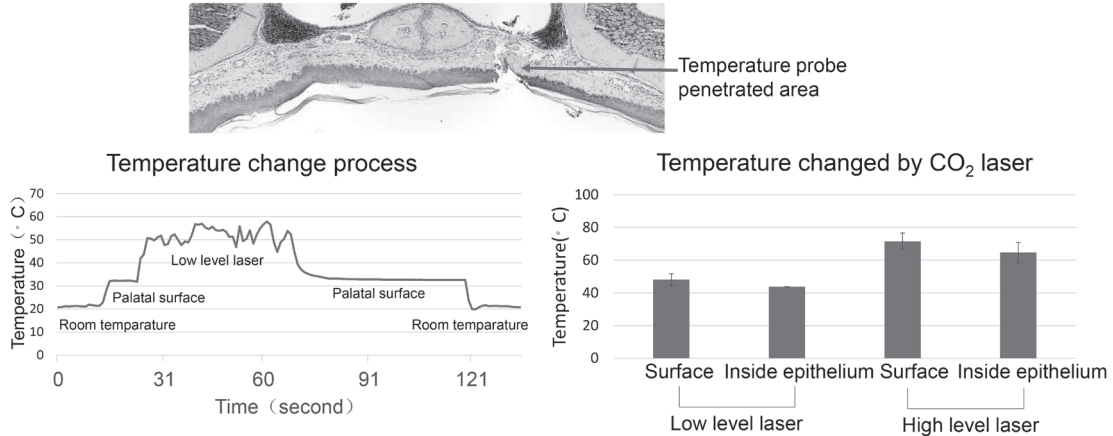
#### Wound Size



In our experiment, the size of wound which was the distance between each epithelial margin (arrowheads) was compared between control and laser groups in day 2 and 3. In both of the low and high CO<sub>2</sub> laser treatment groups, the size of wound was significantly narrowed compared to control group in day 3. The wound area was covered by renewing epithelium in day 5.

# Epithelial recovering of wound significantly faster in CO<sub>2</sub> laser treatment group.

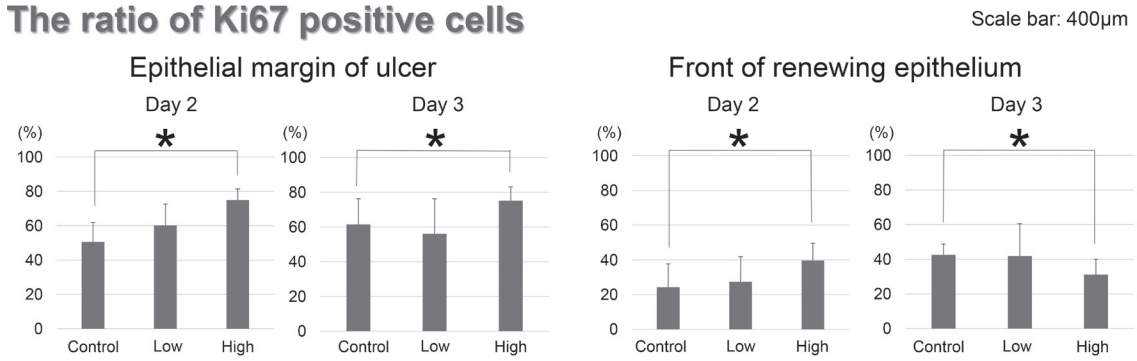
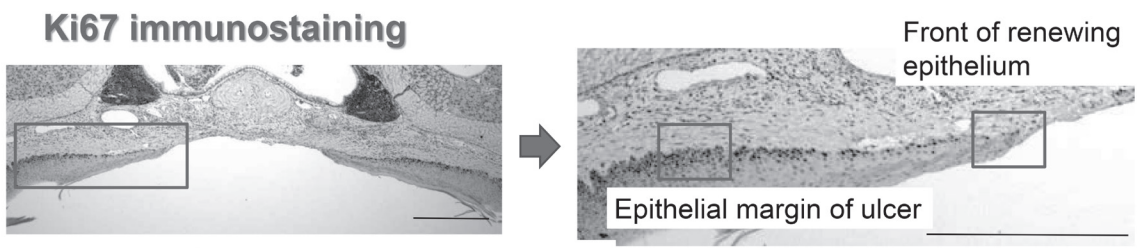
## 2. Temperature change caused by CO<sub>2</sub> laser



Temperature was raised to 45 degree on the palatal surface and 43 degree inside the epithelium by low level laser treatment. For high level laser treatment, the temperature was 72 and 60 degree, respectively.

## 3. Cell proliferation activity in renewing epithelium with/without CO<sub>2</sub> laser treatment

To evaluate the cell proliferation activity, we counted the number of Ki67/hematoxylin positive cells in the front of renewing epithelium and the epithelial margin of ulcer.

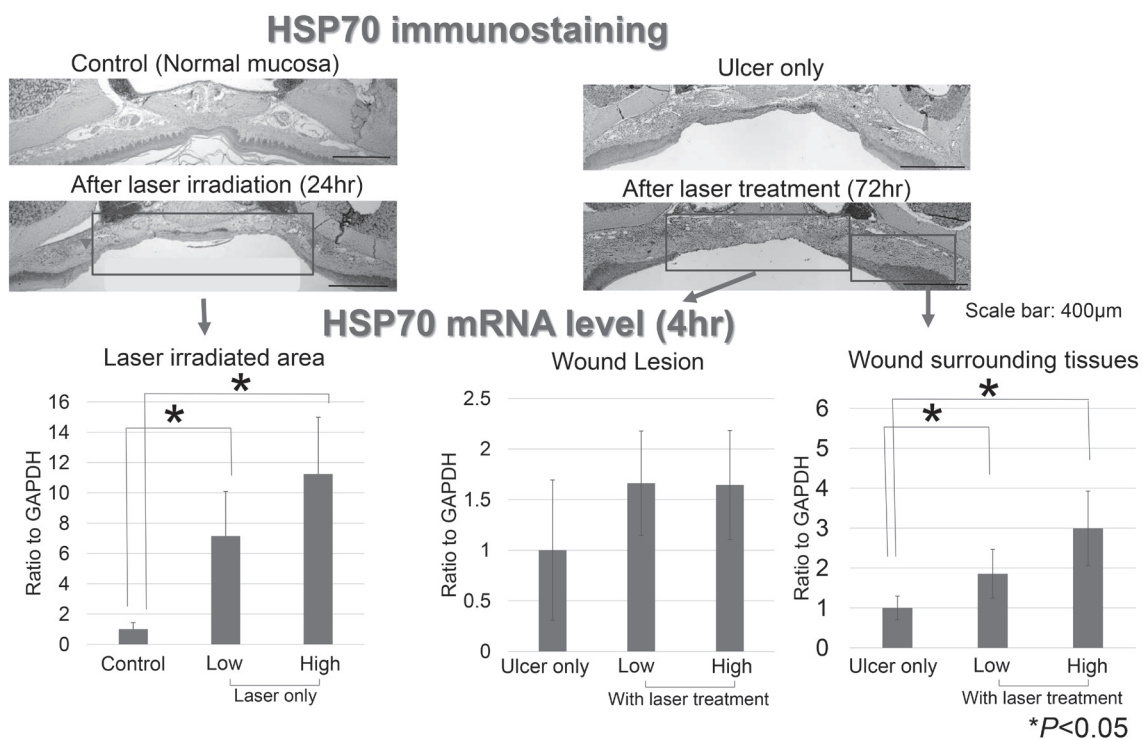


\*P<0.05

In the cell proliferation assay with Ki67 staining, the epithelial cell proliferation of the margin of ulcer in high-CO<sub>2</sub> laser treatment group was significantly higher than that in control group in day 2 and 3. However, in the front of the new epithelium, it was also higher in high-CO<sub>2</sub> laser treatment group in day 2.

**CO<sub>2</sub> laser treatment induced the cell proliferation of epithelium.**

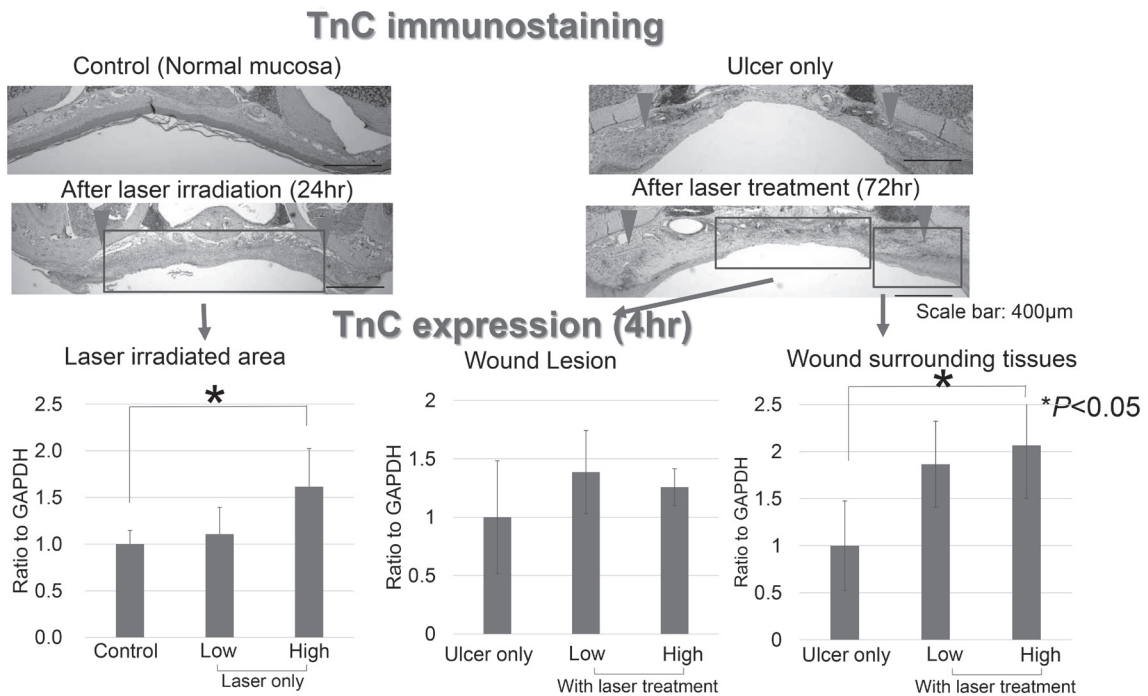
#### 4. HSP 70 expression after CO<sub>2</sub> laser irradiation



HSP70 expressed mainly in epithelial area (arrowheads). After CO<sub>2</sub> laser treatment, TnC mRNA expression was not upregulated in WOUND LESION. However, the expression of HSP70 mRNA in NORMAL MUCOSA was significantly upregulated in low- and high-CO<sub>2</sub> laser groups after CO<sub>2</sub> laser irradiation. Thereby, mucosa surrounding wound lesion was checked after CO<sub>2</sub> laser treatment. The expression of HSP70 mRNA in this area was significantly upregulated in both low- and high-CO<sub>2</sub> laser treatment group.

**CO<sub>2</sub> laser induced HSP70 expression in normal epithelium.**

## 5. Tenascin C expression after laser irradiation



TnC was widely expressed in mesenchyme after CO<sub>2</sub> laser irradiation or treatment (arrowheads). Similar like HSP70 results, the expression of TnC mRNA in wound surrounding area was upregulated after laser treatment..

**CO<sub>2</sub> laser induced TnC expression in normal mucosa.**

## DISCUSSION

According to the results, CO<sub>2</sub> laser treatment can help wound healing of ulcer through inducing the epithelial cells proliferation. HSP70 is the important factor for protecting the cells from some stresses including the temperature, its expression is mainly distributed in the epithelial region. Therefore, it was suggested that CO<sub>2</sub> laser irradiation increased the temperature of the ulcer region as well as the surrounding normal epithelium and accelerated the cell proliferation in these surrounding normal epithelium through induction of HSP70 expression.

In our experiment, TnC expression in lamina propria of wound area was also accelerated. TnC has been known about the key regulator for the cell proliferation and the migration during wound healing. TnC is also possible to be related with faster wound recovering in CO<sub>2</sub> laser treatment. Further experiments are needed to address this point.