Supplementary Data

Table S1. Composition of CO_2-producing powders A and B.

Powder	Succinate (g, mol)	$NaHCO_3$ (g, mol)	Na_2CO_3 (g, mol)
A	11.8 (0.1)	16.8 (0.2)	—
B	11.8 (0.1)	8.4 (0.1)	5.3 (0.05)

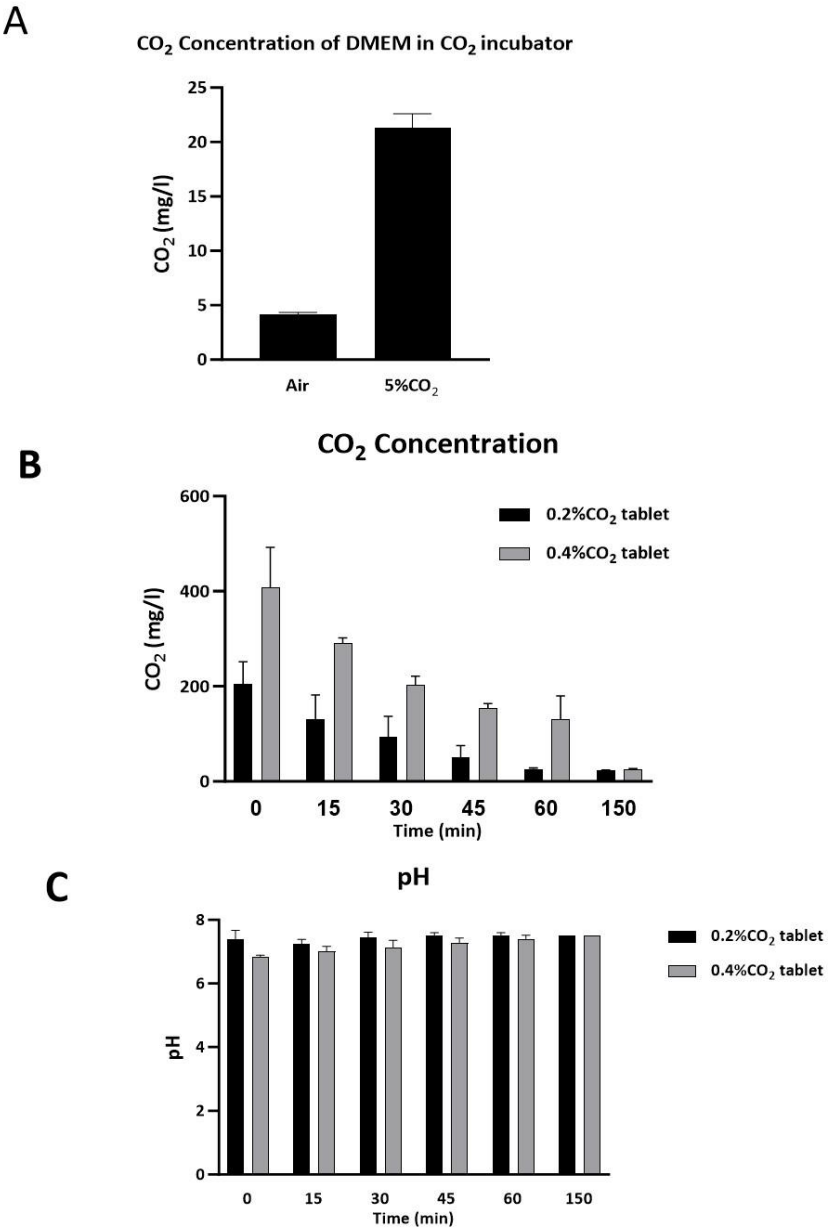
Supplementary Data

Table S2. CO_2 concentration and pH in DMEM dissolved with CO_2-producing powders.

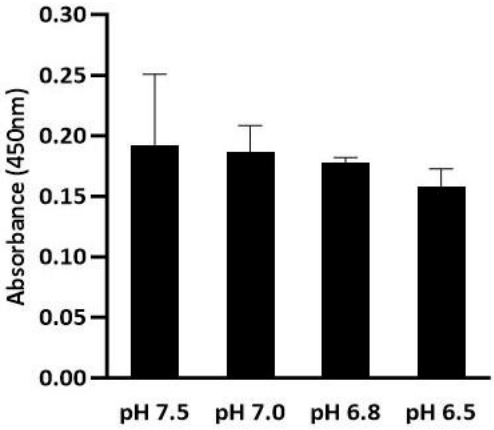
Medium condition*	CO_2 concentration (mg/L)	pH
Powder A	420–437	6.5–6.6
Powder B	406–422	6.4–6.5

*Powders A and B were dissolved in DMEM at a final concentration of 0.4% (w/v).

Supplementary Figure S1. Changes in CO_2 concentration and pH of medium.
DMEM containing 0.2% or 0.4% (w/v) CO_2 tablet was incubated in a 5% CO_2 incubator at 37°C for the indicated periods. (A, B) CO_2 concentration and (C) pH were measured using a Carbon Dioxide Meter (CGP-31; Toa DKK, Tokyo, Japan) and a pH meter (Laura; Horiba, Kyoto, Japan), respectively. Data represent means ± SD (n = 3).

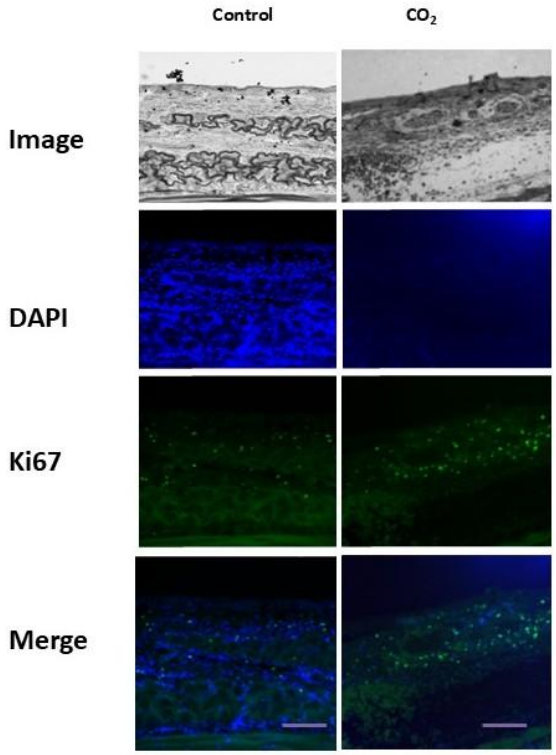


Supplementary Figure S2. Effect of pH on proliferation of human dermal fibroblasts (hDFs). hDFs (5×10^3) were seeded and cultured for 24 h, then treated with DMEM adjusted to the indicated pH values for 20 min. Cells were subsequently incubated in DMEM containing 10% FBS (control medium) for 24 h. Proliferation was determined using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. Absorbance at 450 nm was measured using a microplate reader (EnSpire 2300 Multilabel Reader; PerkinElmer, Waltham, MA, USA). Data represent means ± SD (n = 3).



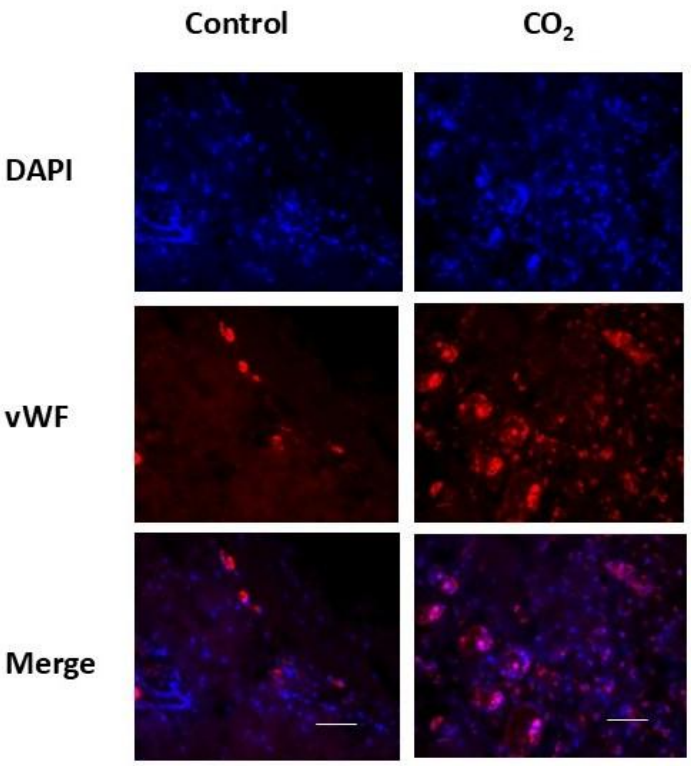
Supplementary Figure S3. Immunohistochemical staining of mouse wound sections following CO_2 bathing. Two circular full-thickness wounds (approximately 6 mm in diameter, including panniculus carnosus) were created on the dorsal skin of mice. One day post-surgery, mice were immersed under anesthesia in 0.5% (w/v) CO_2 tablet-dissolved water at 37°C for 15 min. CO_2 bathing was repeated on day 3, and mice were sacrificed by CO_2 inhalation on day 4. Wounds were excised, fixed in 4% paraformaldehyde (Wako Pure Chemical Industries, Tokyo, Japan), and sectioned at 5 μm. Sections were labeled with anti-Ki67 (Cell Signaling Technology, Danvers, MA, USA) and anti–von Willebrand factor (vWF) (Proteintech, Rosemont, IL, USA).
(A) Representative Ki67 staining showing proliferative cells (scale bar = 100 μm; magnification ×200). (B) Representative vWF staining showing endothelial cells (scale bar = 20 μm; magnification x 400).

A



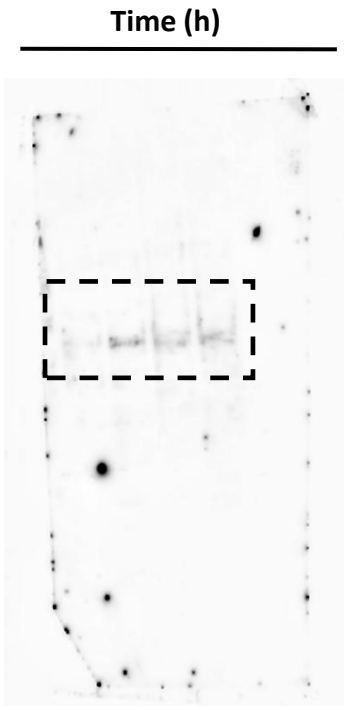
(Continued)

B

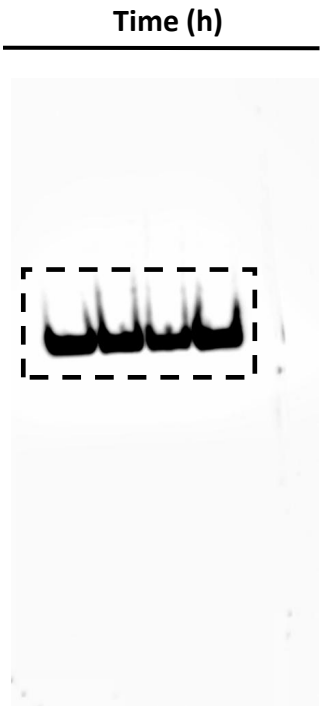


Raw data of western blots

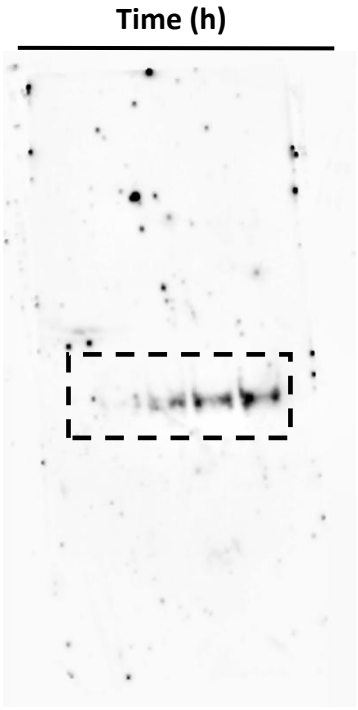
Original immunoblots for figure 5 A of the manuscript showing p-AKT, AKT, p-ERK, ERK, β-Actin expression in the HUVECs.



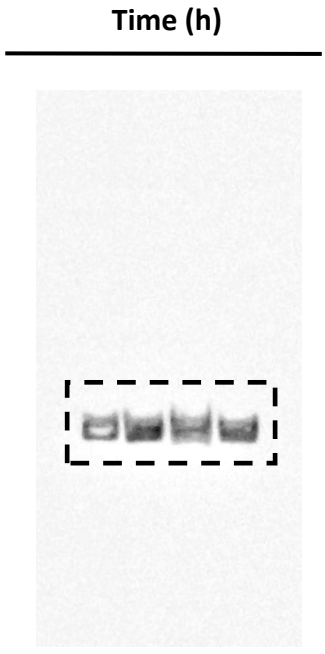
p-AKT



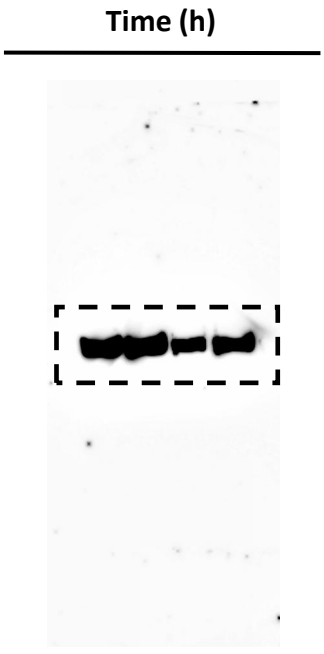
AKT



p-ERK



ERK



β-actin

Original immunoblots for figure 6 C of the manuscript showing p-ERK, ERK, Actin expression in the HUVECs.