Supporting Information

Surfactant-free decellularization of porcine aortic tissue by subcritical dimethyl ether

Hideki Kanda[1, *], Daigo Ando[1], Rintaro Hoshino[1], Tetsuya Yamamoto[2], Wahyudiono[1], Shogo Suzuki[3], Satoshi Shinohara[3], Motonobu Goto[1]

[1] Department of Materials Process Engineering, Nagoya University, Nagoya, 464–8603 Japan

[2] Department of Chemical Systems Engineering, Nagoya University, Nagoya, 464–8603 Japan

[3] Eco Business Development Center, Richo, 1-10 Komakado, Gotenba, 412−0038 Japan

*Corresponding Author:

Email: kanda.hideki@material.nagoya-u.ac.jp; Tel.: +81–52–789–3990

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DME is used in food processing and is not problematic for food consumption. However, it has not been evaluated for its toxicity to cell culturing in the past. This is because DME has a very low boiling point and volatilizes at room temperature and atmospheric pressure, so its residues are very low. However, the decellularized aorta created in this study will be examined as a three-dimensional scaffold by culturing cells in future studies. If trace amounts of DME gas were to remain in decellularized tissue, it is expected that DME gas would dissolve into the culture medium during subsequent cell culture.

Therefore, this study investigates the biological impacts of DME-dissolved water by bioassay using *Micrococcus luteus* as the indicator microorganism. This microbe is commonly detected in soil, water, and mammalian skin as part of the normal microbiota. [49–52] In other words, it is difficult to use subcritical DME as a lipid extraction solvent if DME gas is toxic to this microorganism.

The purified water used for the bioassays was LC/MS grade purchased from Fujifilm Wako Pure Chemicals (Osaka, Japan) and was de-oxygenated by bubbling DME gas. 10 cm^3 Purified water and 10 cm^3 subcritical DME were sealed in a 96 cm^3 pressure-resistant glass container covered with polycarbonate (HPG-96-3, Taiatsu Techno Corp., Saitama, Japan) and shaken for 10 minutes at 20°C, 40°C and 60°C in a water bath. These different temperatures setting is based on the assumption that lipids may be extracted using subcritical DME at various extraction temperatures in a practical use. This volume ratio of water to subcritical DME is in a state of partial mixing at all temperatures, so the water is saturated with DME. After that, the pressure in the pressure-resistant glass vessel was reduced to atmospheric pressure by opening a release valve, and water saturated by DME gas (DME-dissolved water) was obtained in the pressure-resistant glass vessel. The DME-dissolved water was filtered through a filter paper (DISMIC-25AS, 25AS020AS, pore size 0.20 μm, Advantec, Tokyo,

Japan) to remove impurities.

The antibacterial activities of the DME-dissolved water against *M. luteus* were evaluated by the agar well diffusion method. [53] The composition of the bioassay plate has been described by Arakawa *et al.* [54] The bioassay plate had two layers: a bottom layer, consisting of a tryptic soy broth (TSB; Difco Laboratories) with 1.5% agar, and a top layer, consisting of TSB with 0.8% agar supplemented with 2% M. luteus-filled growth suspension. Subsequently, a hole with a diameter of 8 mm was bored in each agar plate using a sterile cork borer. Polymer samples (8 μL) were placed in the agar wells and then incubated at 28 °C for 48 h. The antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding the DME-dissolved water. [52,55]

The effect of each DME-dissolved water was examined via bioassays; the results obtained are presented in Figure S1. No inhibition zone was formed for all DME-dissolved water, as shown in Figure S1. In other words, DME gas dissolved in water is not toxic to *M. luteus*. Thus, concerns about DME for cell culture have been allayed by this experiment, and there is hope for the culture of animal cells using the decellularized tissue prepared in this study.

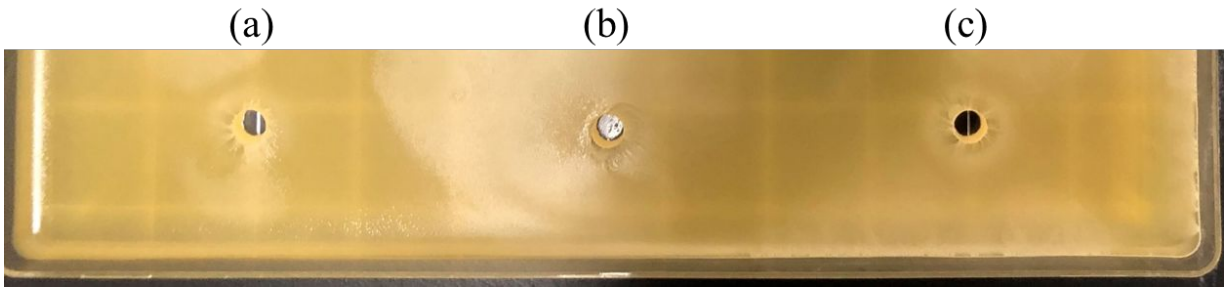

Figure S1. Results of the bioassays of DME-dissolved water at (a) 20 °C, (b) 40 °C, (c) 60 °C.

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