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Fabrication of PDMS-Based Micro-Chambers Designed to Have Optimal Gas-Permeability for Plant Cell Culture

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Objectives

In this paper, we propose a polydimethylsiloxane (PDMS)-based cell culture micro-chamber that is designed to have controllable gas permeability which is vital factor in plant cell culture.

Experiments

- Fabrications

The schematic of the PDMS-based cell culture chamber is illustrated in Figure 1(a), and it is fabricated by the process shown in Figure 1(b). The 10:1 mixture of the PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning, Midland, MI) was poured onto the master mold placed in a Petri dish. The mixture was thermally cured for 2 hours on a hot plate at 80°C, and separated from the mold. Then, the cylindrical culture chamber was produced. Figure 1(c) illustrates both the scanning electron microscopy (S-4300, Hitachi, Japan) of the SU-8 master mold and the fabricated PDMS based culture chamber.

- Measurement

Evaporation Rate (%) = $((M_i - M_s) / M_c) \times 100$

M_i : Initial weight of culture chamber

M_s : The weight of culture chamber after evaporation

M_c : The initial weight of culture medium

- Cell preparation & Culture

Protoplasts of *Nicotiana tabacum* were isolated according to a modification of Sun's method.

Results , Discussion and Conclusion

We have designed and fabricated a PDMS-based culture chamber for plant cells. We suggest a method satisfying two requirements: good gas supply and less evaporation of medium. For the good gas supply, the optimal thickness of a PDMS cover sheet was determined by the experiments. The excessive evaporation of medium decreased through the maintenance of the surrounding humidity of the culture chamber at 85%. To evaluate the function of the culture chamber, we cultured the protoplasts of *Nicotiana tabacum*, which, after being divided, survived successfully for 10 days. Our design approach can be extended to the design of culture devices for other cells (e.g., human hepatocyte or cancer cells).

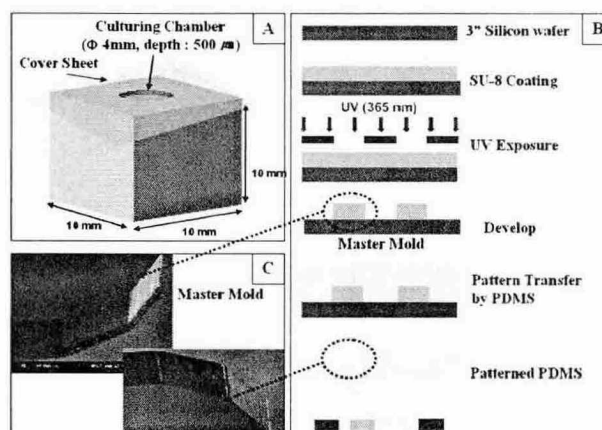


Figure 1. (a) Schematic of PDMS-based cell culturing chamber, (b) fabrication process of culturing chamber—patterns of culturing chambers on the SU-8 master mold are transferred to the PDMS, (c) SEM image of SU-8 master mold (upper picture) and of PDMS-based culturing chamber