Bacterial Isolation Microwell-Plug (µWELLplug) for Rapid Antibiotic Susceptibility Testing Using Morphology Analysis

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The photolithography system used in this experiment is maskless equipment. Thus, it can easily fabricate various microwell patterns by controlling the photomask files (image files). Distinguishing between one microwell pattern and another becomes confusing over time. However, that problem can be solved by changing the shape or controlling the size, as shown in **Figure S1**.



Figure S1. Fabricating microwell patterns of various sizes and shapes. (scale bar: 200µm)

To estimate the degree of protrusion or indentation on the Bacterial Isolation Microwell-Plug (μ WELLplug), we assume that the external appearance is elliptical, spherical, or cylindrical as shown **Figure S2a**. In the case of an elliptical shape, it is possible to predict the degree of protrusion or indentation as described in the main text. For a spherical appearance, we could predict the extent using the following equations (1), (2), and (3). For a cylindrical shape, the degree can be predicted using equation (4).



The calculated results are shown in **Figure S2b**, and it was confirmed that the fabricated hydrogels appeared most similar to the elliptical shape.



Figure S2. Analysis of the appearance of the Bacterial Isolation Microwell-Plug (μWELLplug).(a) Three types of appearance, elliptical, circular, and cylindrical shapes, were predicted. (b) Comparative analysis of the experimental results.

The bubble holes not only eject air bubbles trapped between the well plate and the Bacterial Isolation Microwell-Plug (μ WELLplug), but can also be used to change the environment in the space. Depending on the size of the bubble hole, it is possible to insert a thin needle or a thick pipette tip, so researchers can choose the hole design that suits their needs. By using a pipette tip, an attempt was made to refresh the medium inside the space between the well plate and the μ WELLplug, as shown in **Figure S3**.



Figure S3. (a) A Bacterial Isolation Microwell-Plug (μ WELLplug) and culture medium in a 96well plate. (b) Inserting a suction tool of a size compatible with the design, such as a pipette tip or needle. (c) Empty space after removing the internal medium using the inserted suction tool. (d) Filled space after discharging fresh medium in the empty space (scale bar: 500 µm).



Figure S4. Analysis of area contributed to cell isolation conducted with 30 μ WELLplugs. Cell isolation is mainly possible at the edge since strong pressing is implemented at the edge. 34% of the total area contributed to press against the bottom floor, cell isolation, on average (scale bar: 1 mm).



Figure S5. Analysis of relative population of bacteria in case of methicillin-resistant *Staphylococcus aureus*. The decision criteria set to 2.0 by ampicillin in previous test.