CLAIMS

1. A spheroid-producing device comprising:

a first surface;

a second surface, the second surface being a back side surface of the first surface; and

a plurality of wall surfaces configured to constitute a plurality of holes penetrating through the first surface and the second surface, wherein an equivalent diameter of inscribed circles of openings in the first surface is greater than an equivalent diameter of inscribed circles of openings in the second surface,

wherein a width between one of the wall surfaces constituting one of the holes and another one of the wall surfaces constituting another adjacent one of the holes is 2 mm or less, and the width is a width of an end portion that is close to the first surface.

2. The spheroid-producing device according to Claim 1, wherein the equivalent diameter of the inscribed circles of the openings in the second surface is within a range of 200 micrometers to 2mm.

3. The spheroid-producing device according to Claim 1 or 2, wherein at least portions of the respective wall surfaces include inclinations with an angle θi greater than one degree and smaller than 90 degrees with respect to the second surface.

4. The spheroid-producing device according to any one of Claims 1 to 3, wherein

a culture medium is poured into the respective holes from the first surface,

when a contact angle θc between surfaces of a material forming the respective wall surfaces and the culture medium is within a range of $-1<\cos\theta c\leq 0$, an equivalent radius Rout (cm) of circumscribed circles, which is a half of an equivalent diameter of the circumscribed circles of the openings in the second surface, is less than or equal to a value of a variable X that is defined by an equation of

$(2/3)\alpha X^2+pX=2\gamma L$,

where p is a water pressure [g/cm^2] at the openings in the second surface, α is a specific gravity of the culture medium, and γ_L [g/cm] is surface tension of a liquid.

5. The spheroid-producing device according to any one of Claims 1 to 3, wherein

the culture medium is poured into the respective holes from the first surface, and

when the contact angle θc between surfaces of a material forming the respective wall surfaces and the culture medium is within a range of $0<\cos\theta c<1$, the equivalent diameter of the inscribed circles is designed so that equations of

$\gamma_L \cos\theta c - \gamma_S > 0$

and

$p = \gamma L \times (2/r)$

are satisfied, where p is a water pressure [g/cm^2] at the openings in the second surface, γ_L is surface tension of a liquid [g/cm], r is a radius of curvature [cm], and γ_L is surface tension of the liquid [g/cm].

6. The spheroid-producing device according to Claim 3, wherein the two wall surfaces incline at the angle θi with respect to the second surface in the end portion.

7. The spheroid-producing device according to any one of Claims 1 to 6, wherein the spheroid-producing device is a resin molding made of one of or a combination of acrylic resin, polylactic acid, polyglycolic acid, styrene resin, acrylic-styrene copolymer resin, polycarbonate resin, polyester resin, polyvinyl alcohol resin, ethylene-vinyl alcohol copolymer resin, thermoplastic elastomer vinyl chloride resin, silicone resin, and silicon resin.

8. The spheroid-producing device according to Claim 7, wherein functional groups are formed on the plurality of wall surfaces by the surface modification treatment, which includes one of or a combination of plasma treatment, corona discharge, and UV ozone treatment.

9. The spheroid-producing device according to Claim 7, wherein the plurality of wall surfaces are coated with a substance made of one of or a combination of inorganic substances, metal, synthetic polymers, dimers, trimers, tetramers, and biobased polymers.

10. The spheroid-producing device according to any one of Claims 8 and 9, wherein front surfaces of the plurality of wall surfaces include nanometer order microstructures.

11. The spheroid-producing device according to any one of Claims 1 to 6, wherein the spheroid-producing device is a molding made of one of or a combination of inorganic substances such as metal and glass.

12. The spheroid-producing device according to Claim 11, wherein surfaces of the plurality of wall surfaces are modified by the surface modification treatment, which includes one of or a combination of plasma treatment, corona discharge, and UV ozone treatment.

13. The spheroid-producing device according to Claim 11 or 12, wherein the plurality of wall surfaces are coated with a substance made of one of or a combination of inorganic substances, metal, polymer, diners, trimers, and tetramers.

14. The spheroid-producing device according to any one of Claims 11 to 13, wherein the front surfaces of the plurality of wall surfaces include nanometer order microstructures.

15. A method for recovering spheroids comprising bringing the second surface of the spheroid-producing device according to any one of Claims 1 to 14 into contact with a solution selected from water, a culture medium, and a buffer solution in order to recover spheroids present in each of a droplet formed at the respective holes.

16. A method for recovering spheroids comprising applying a pressure on the first surface of the spheroid-producing device according to any one of Claims 1 to 14 in order for spheroids present in each of a droplet formed at the respective holes to be flown out of the openings in the second surface.

17. A method for producing spheroids using the spheroid-producing device according to any one of Claims 1 to 14, the method comprising:

pouring a culture medium containing cells into the respective holes from the first surface;

forming droplets at the respective holes; and

culturing the cells in the droplets in order to produce spheroids from the cells in each of the droplet.

18. The method according to Claim 17 further comprising bringing the second surface into a solution selected from one of water, a culture medium, and a buffer solution in order to recover the spheroids from each of the droplet.

19. The method according to Claim 17 further comprising applying a pressure on the first surface to thereby destroy the droplets and to cause the spheroids to be flown out of the openings in the second surface.

20. A method for recovering spheroids from a spheroid-producing device, which comprising:

a first surface;

a second surface, the second surface being a back side surface of the first surface; and

a plurality of wall surfaces configured to constitute a plurality of holes penetrating through the first surface and the second surface, wherein an equivalent diameter of inscribed circles of openings in the first surface is greater than an equivalent diameter of inscribed circles of openings in the second surface, wherein

the method comprising,

applying a pressure on the first surface in order for spheroids present in each of a droplet formed at the hole to be flown out of the openings in the second surface,

21. A method for producing spheroids using a spheroid-producing device, wherein the spheroid-producing device comprising:

a first surface;

a second surface, the second surface being a back side surface of the first surface; and

a plurality of wall surfaces configured to constitute a plurality of holes penetrating through the first surface and the second surface, wherein an equivalent diameter of inscribed circles of openings in the first surface is greater than an equivalent diameter of inscribed circles of openings in the second surface, and

the method comprising:

pouring a culture medium containing cells into the respective holes from the first surface;

forming droplets at the respective holes;

culturing the cells in the droplets in order to produce spheroids from the cells in each of the droplets; and

applying a pressure on the first surface to thereby destroy the droplets and to cause the spheroids to be flown out of the openings in the second surface.

22. The spheroid-producing device according to Claim 1, wherein

the openings in the first surface faces to a space arranged above the spheroid-producing device,

a droplet is formed from a culture medium in each of the hole by a pressure and

surface tension of the culture medium, when the culture medium is poured into each of the hole and the space, and

the droplet is formed at the opening in the second surface and does not come around the second surface or the droplet is formed halfway down the hole.

23. The spheroid-producing device according to Claim 22, further comprising a well container providing the space.

SPHEROID-PRODUCING DEVICE, METHOD FOR RECOVERING SPHEROIDS, AND METHOD FOR PRODUCING SPHEROIDS

ABSTRACT

The present invention provides a device for producing a large number of uniform spheroids by an easy method. The spheroid-producing device (1) at least includes a first surface (11), a second surface (12), and a plurality of wall surfaces (13). The second surface (12) faces the first surface (11). The respective wall surfaces (13) constitute a plurality of holes penetrating through the first surface and the second surface. In addition, an equivalent diameter of inscribed circles of openings in the first surface (11) is greater than an equivalent diameter of inscribed circles of openings in the second surface (12).

Fig. 2 accompanies the abstract.