

Title: *In vivo* and *ex vivo* methods of growing a liver bud through tissue connection

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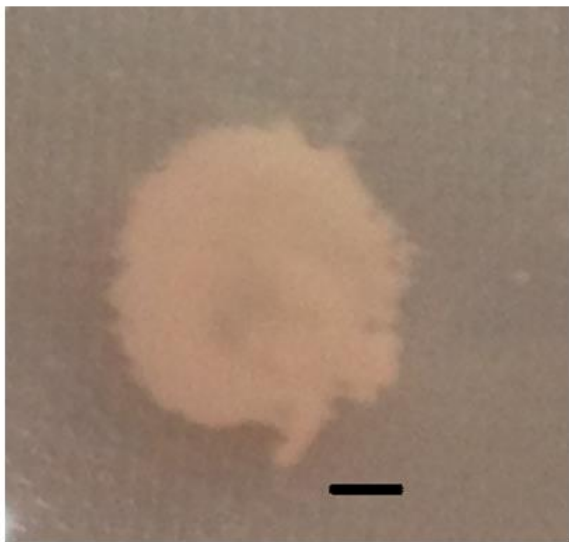
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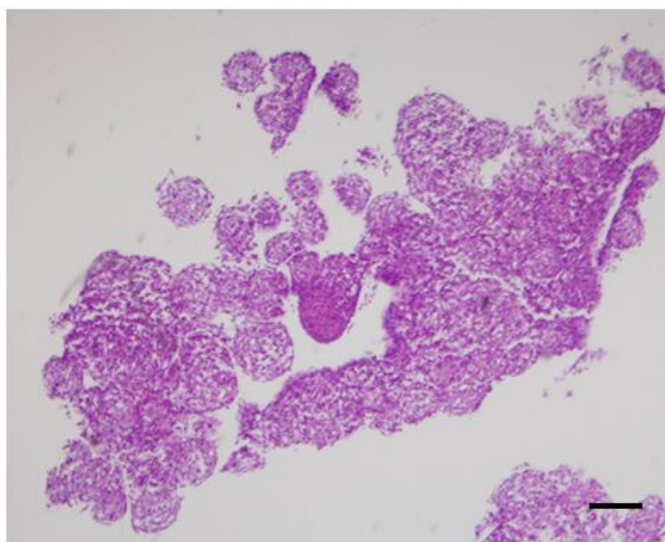
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Supplementary Information

a

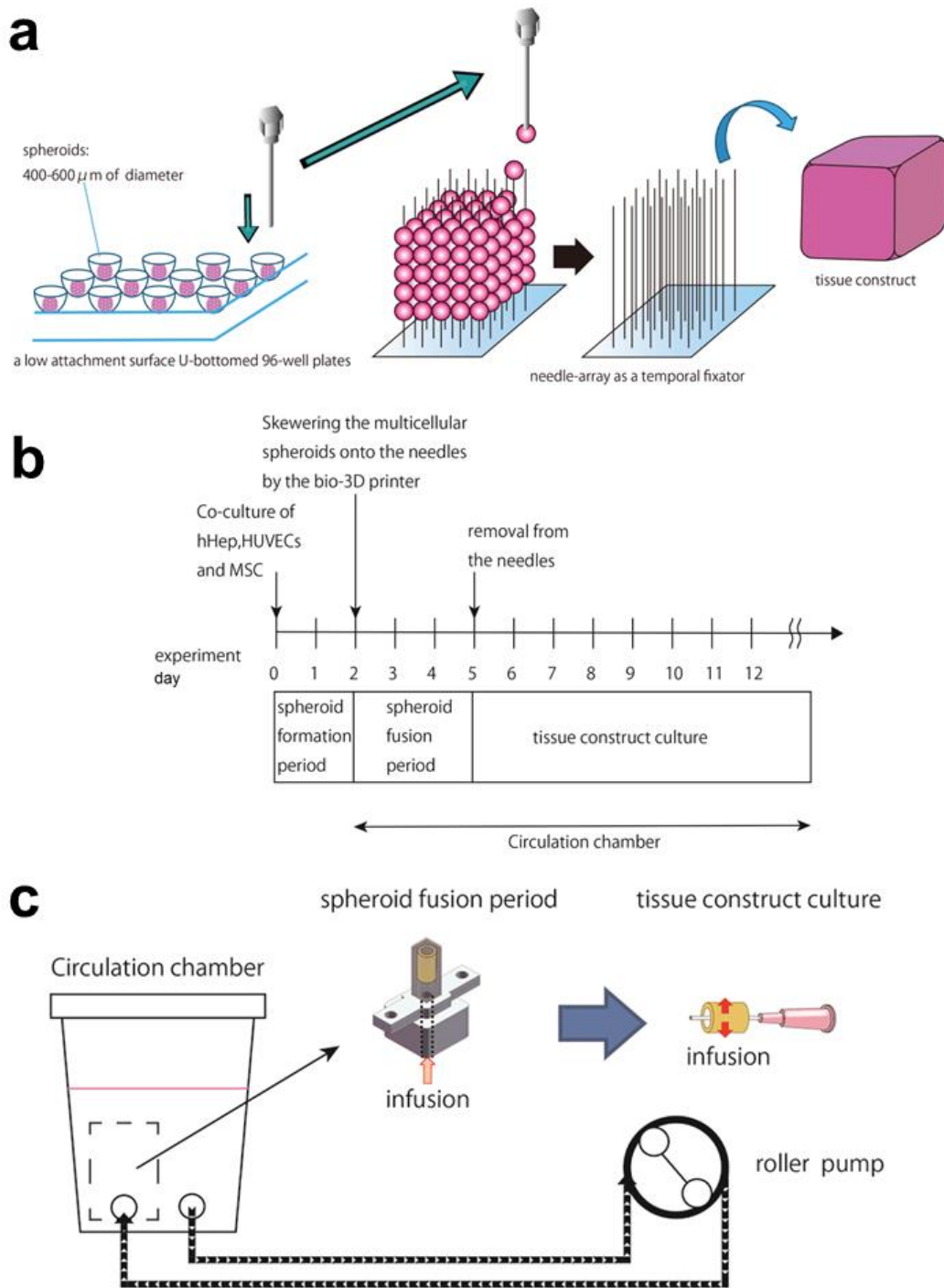


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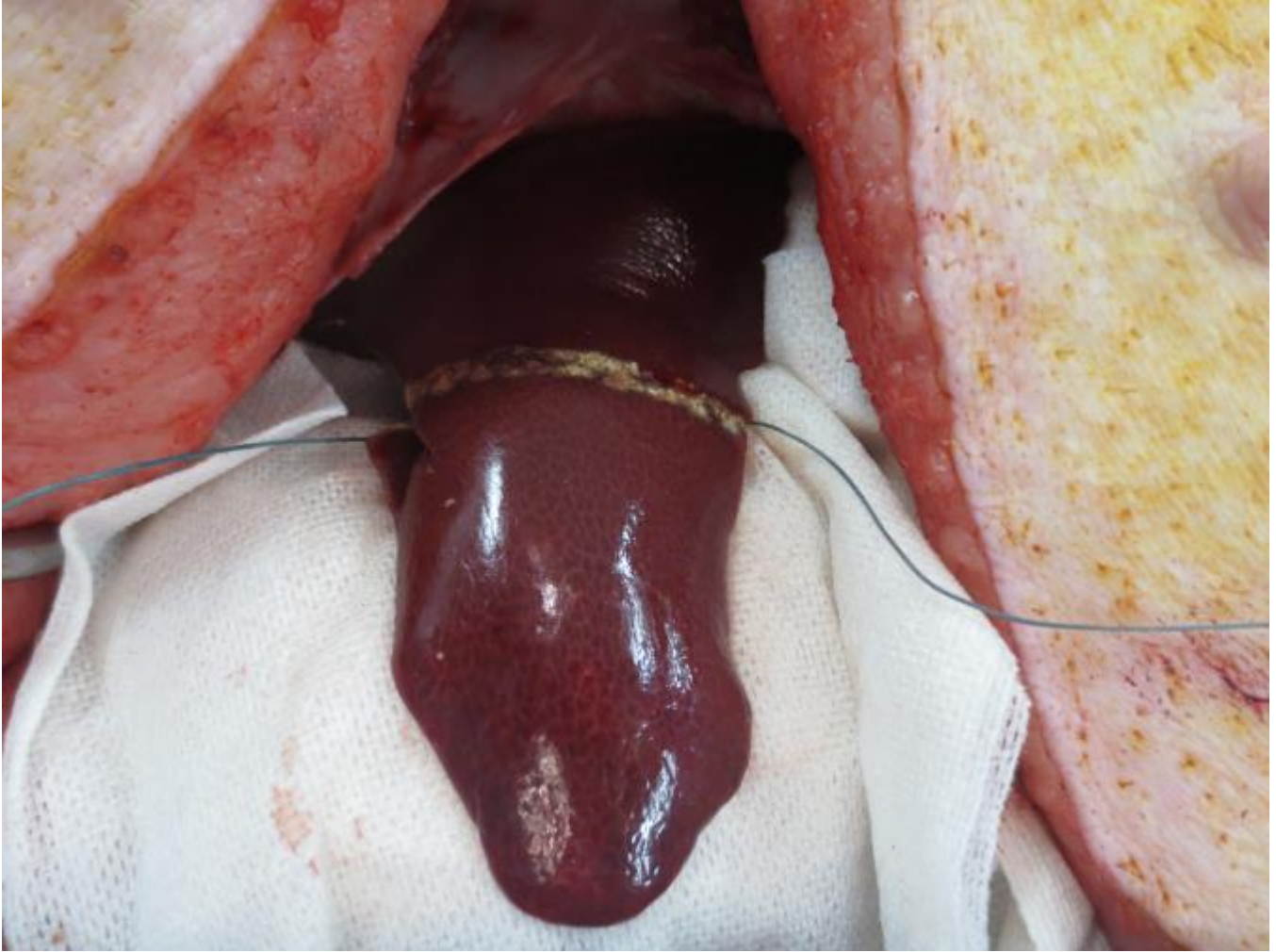
Supplementary Figure 1. Scalable tissue fabricated using the unarranged fusion of spheroids.

(a) Gross observations of scalable tissue formed by the parallel fusion of small spheroids in a low attachment dish. Scale bar, 250 μm . (b) An HE-stained cross section of the tissue. Scale bar, 200 μm .

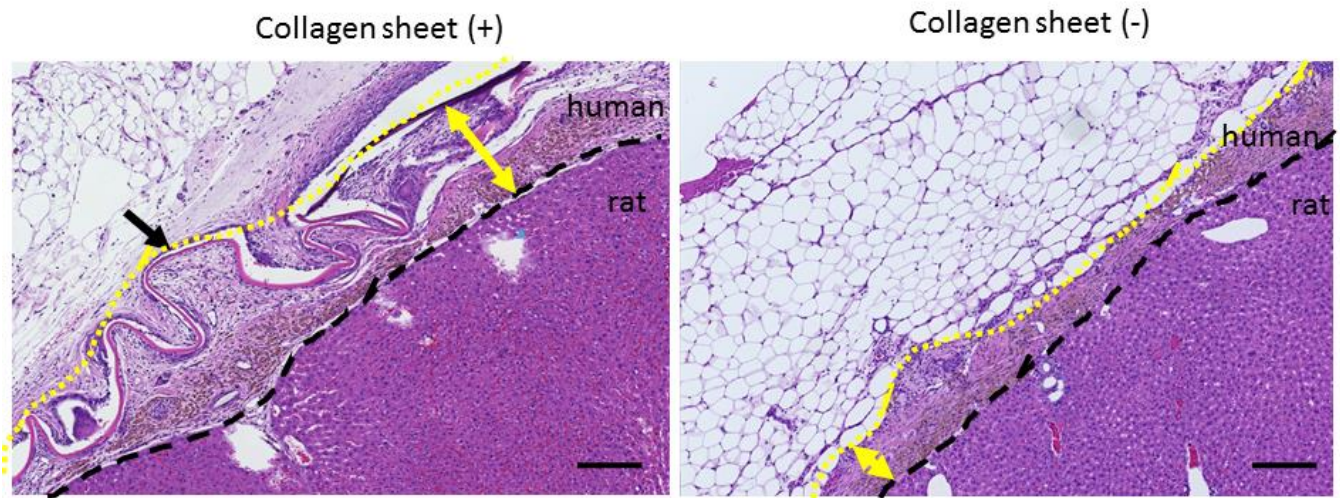


Supplementary Figure 2. The experimental strategy used to construct liver-like tissue with a new 3D bioprinter.

(a) A schematic representation of our 3D bioprinter. (b) The timetable for constructing the liver tissue from liver bud-like spheroids *in vitro*. (c) The circulation system for culturing 3D tissue.



Supplementary Figure 3. The creation of an intact transection plane of the liver in a micro mini pig.



Supplementary Figure 4. Comparison of the fibrotic plaque surrounding the transplant with or without the covering with a collagen sheet.

An HE-stained cross-section on the 28th day after transplantation. The pink sheet indicated by the black arrow was the collagen sheet. The black dashed line indicates the border between the rat liver and the transplanted 3D liver tissue. The yellow dashed line indicates the border between the transplanted 3D liver tissue and omentum. The yellow two-way arrow indicates the thickness of the fibrotic plaque in the transplanted tissue. Scale bar, 100 μm .

Supplementary Table 1.

[TaqMan Probe]

18S; Eukaryotic 18S rRNA

Product ID: Hs99999901_s1

CK18; KRT18; keratin 18

Product ID: Hs02827483_g1

TAT

Product ID: Hs00356930_m1

HNF4A; hepatocyte nuclear factor 4 alpha

Product ID: Hs00230853_m1

ALB: albmin

Product ID: Hs00910225_m1

[SYBR Green Primer]

TDO2; tryptophan 2,3-dioxygenase

Forward : ggccatgtcagagatgaaagga

Reverse : gtccaaggctgtcatcgtct

Number : NM_005651.3

364~483→120bp

CYP7A1; Cholesterol 7 alpha-hydroxylase

Forward : agtcagcttgaaggcaatc

Reverse : tcctccttagctgtccggat

Number : NM_000780.3

1038~1169→132bp

Supplementary Video 1. Orthotropic transplantation method on the transected parenchyma.

An intact parenchyma at the transection plane was created by ligating and cutting the parenchyma with a surgical suture in a rat; hemostasis was achieved by the concurrent ligation of the vessels.

Supplementary Video 2. Physical strength of the ex vivo-fabricated liver tissue.

The *ex vivo*-fabricated liver tissue on the 7th day in culture was strong enough to tolerate physical manipulation