Supporting Information

Parathyroid-on-a-Chip Simulating Parathyroid Hormone Secretion in Response to Calcium Concentration

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1. Validation of PT-r cell

PT-r cell line, which was established from the rat parathyroid gland,[1] was cultured in DMEM with 10% FBS, 100 μg/mL streptomycin, and 100 U/mL penicillin. SFig 2(a) shows the morphology of PT-r cells, and it was observed that PT-r cells expressed PTH and CaSR as shown in SFig 2(b). Organoids were not formed with PT-r cells alone but were formed as an aggregation model. However, PT-r/fibroblast organoids could be fabricated through the co-culture of fibroblasts at an appropriate ratio as shown in SFig 2(c). Both PT-r cell and fibroblast contributed to the organoid formation, and it was confirmed through cell membrane staining as shown in SFig 2(d). The expression of PTH and CaSR in PT-r/fibroblast organoids was observed through immunofluorescence staining as shown in SFig 2(e). The organoids were formed with diameters of 86.4 μm and 107.1 μm from 112.6 cells and 481.1 cells, respectively, as shown in SFig 2(f). PT-r cells cultured in 2D did not secrete PTH, while the PT-r/fibroblast organoids secreted a very small amount of PTH, measured at 2.9 ± 0.7 pg/ml as shown in SFig 2(g). Although the cell line (PT-r cells) expressed PTH and CaSR, it lacked the functionality of PTH secretion, making it insufficient to represent the function of the parathyroid gland.

2. Rat-derived primary cell culture

Rats were photosensitized using 5-aminolevulinic acid hydrochloride (5-ALA), and the parathyroid glands were surgically removed and collected as shown in SFig 3(a). parathyroid glands were digested in DMEM/F-12 containing 205 U/mL collagenase type I for 30 min at 37°C. The diameter of rat parathyroid glands was 1.15 mm as shown in SFig 3(b), and 5 x 10^4 cells were collected from 17 rat parathyroid glands. Cells were cultured in DMEM/F-12 containing 10% FBS, 1 mM $CaCl_2$, 0.7 mM

$MgSO_4$, 100 µg/mL streptomycin, 100 U/mL penicillin, 5 µg/ml Insulin, 5 µg/ml Holo-transferrin, 1% non-essential amino acids, and 2 mM L-Glutamine. The culture medium was changed twice a week. No cell proliferation was observed for 2 weeks culture period on both 2D (culture flask) and 3D (low-attach well plate) methods as shown in SFig 3(c). Parathyroid organoids were formed after 6 days using the 3D culture method, but they dispersed again after 13 days.

3. *In vitro* culture of parathyroid cells

Due to limitations in obtaining normal human parathyroid cells, studies have been reported to establish parathyroid cell lines.[1–3] However, PT-r cell, which is the commercially available parathyroid cell line, did not secrete PTH. Additionally, the culture of primary parathyroid cells is known to be very difficult because of their high level of differentiation.[4] In this study, we faced limitations in obtaining an adequate number of primary parathyroid cells due to their limited proliferation. Therefore, obtaining parathyroid cells from stem cells is considered the most efficient approach for parathyroid research, surpassing the use of cell lines or primary cells. In summary, the cell line has lost its parathyroid gland function, and primary cells possess parathyroid functionality but are difficult to culture. On the other hand, stem cells not only provide an ample cell supply but also readily differentiate into parathyroid cells (STable 1).

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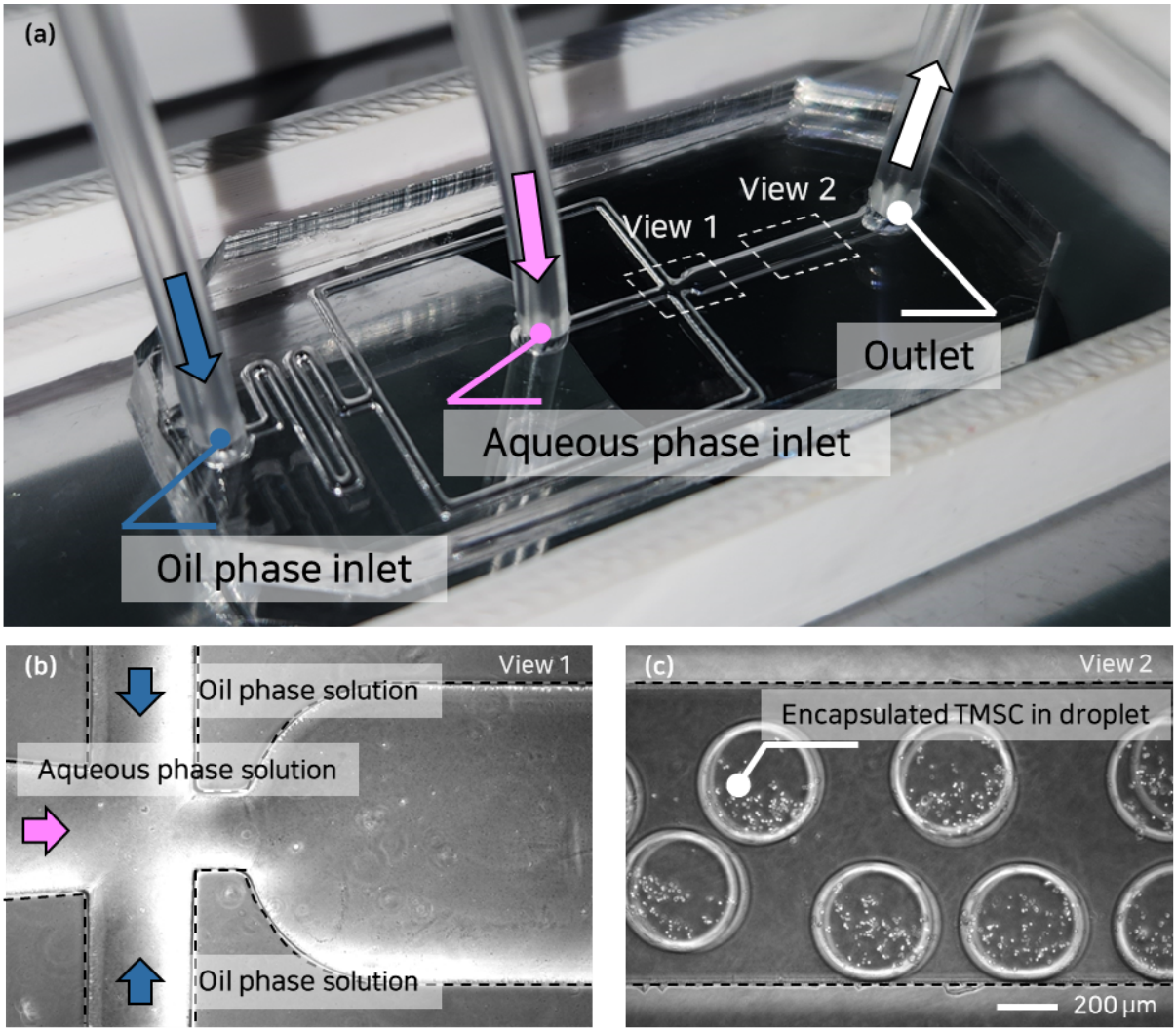
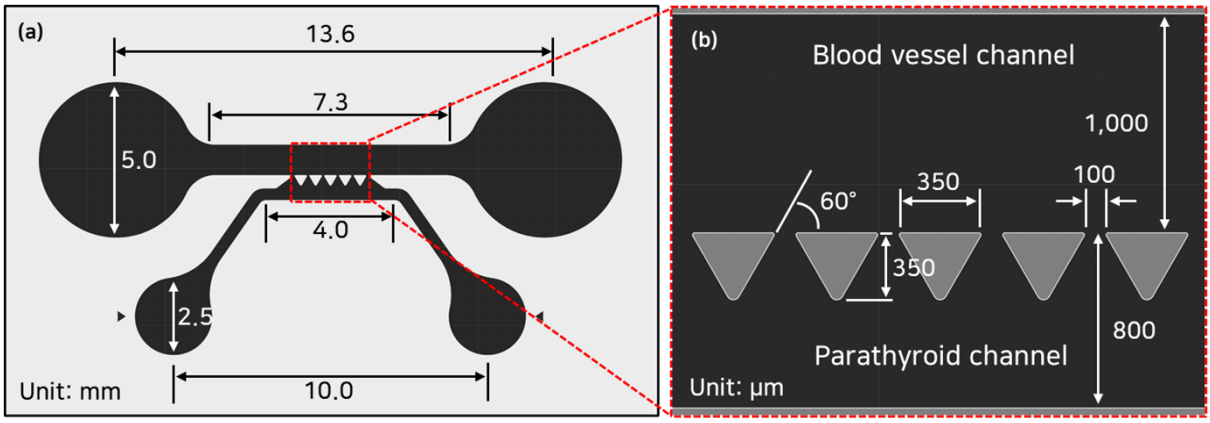
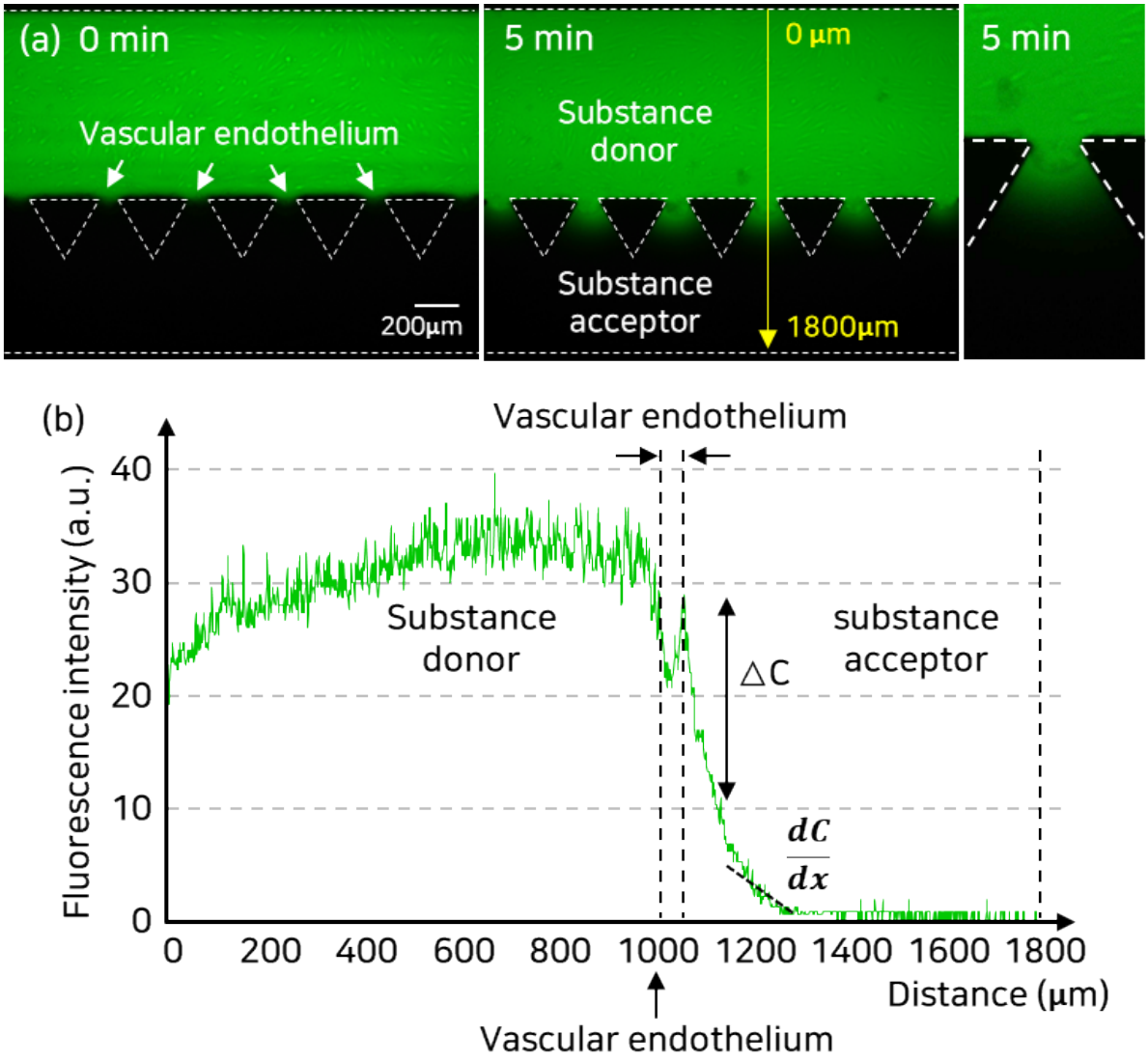


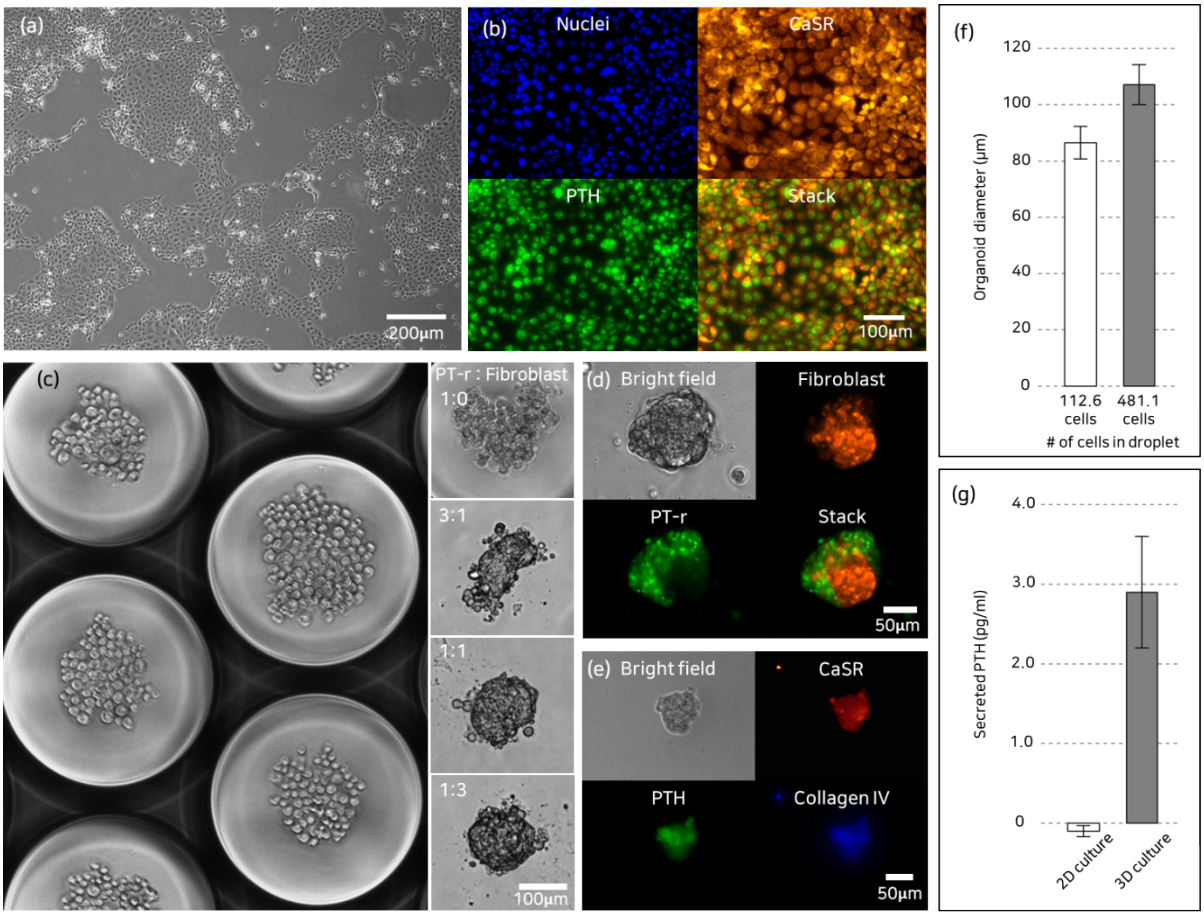
SFig 1(a) Optical image of a droplet-based microfluidic system. This system consists of two inlets for the oil phase solution and the aqueous phase solution injection, and one outlet for collecting droplets. (b) droplet junction where the two fluids meet to form droplets. (c) TMSC encapsulated within the droplet.



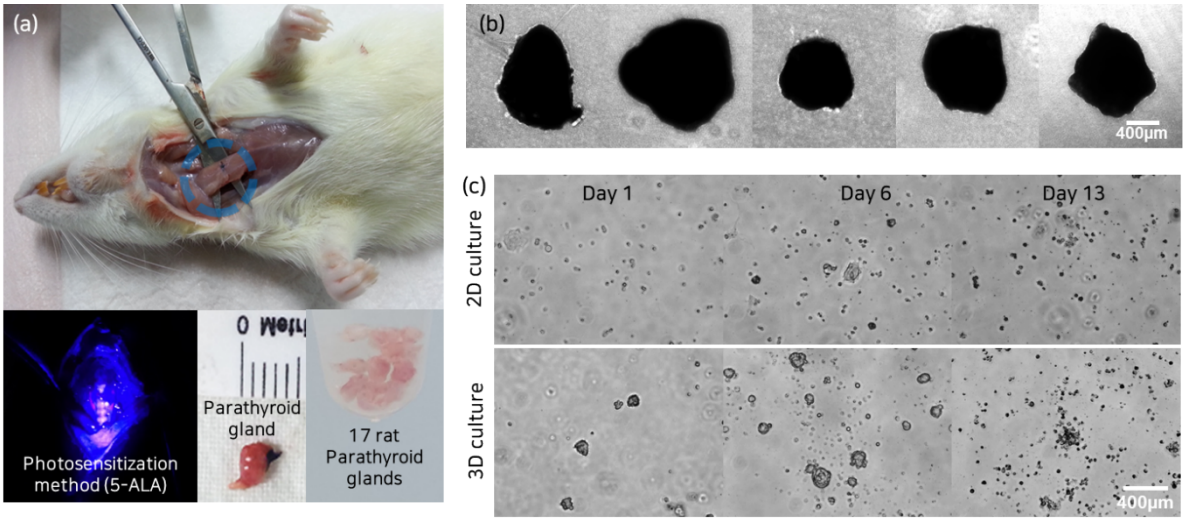
SFig 2(a) The overall structure of parathyroid-on-a-chip. (b) The detailed dimensions of the core region of this chip, are divided into blood vessel channel and parathyroid channel.



SFig 3(a) Injected dextran into the vascular channel. (b) measured fluorescence intensity along the detection line (yellow line).



SFig 4(a) Morphology of PT-r cell. (b) Immunofluorescence staining result for Nuclei (blue), PTH (green), and CaSR (orange) of PT-r cell. (c) Encapsulated PT-r cell and fibroblast in the droplets. (d) Cell membrane-stained PT-r cell (green) and fibroblast (orange) of PT-r/fibroblast organoid. (e) Immunofluorescence-stained PT-r/fibroblast organoid. (f) PT-r/fibroblast organoid size respective to the number of cells. (g) PTH secretion by 2D and 3D cultured PT-r cell.



SFig 5(a) photosensitized rat and extracted rat parathyroid glands. (b) Microscopically observed parathyroid gland specimen. (c) Two-dimensional and three-dimensional cultured rat parathyroid cells.

STable. 1. *In vitro* culture of parathyroid cell and functionality

	Cell line (PT-r cell)	Primary cell		TMSC
Origin	**Rat parathyroid**	**Rat parathyroid**	**Bovine parathyroid**	**Human tonsil**
***In vitro* culture difficulty**	**Low**	**High**	**High**	**Low**
PTH expression	+	**n/a**	+	+
CaSR expression	+	**n/a**	+	+
PTH secretion	-	**n/a**	+	+
[Ca^{2+}] response	-	**n/a**	+	+
Immune rejection for human transplantation	**High**	**High**	**High**	**Low**
Reference	1,2,5	4	4,6	7–9