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ORIGINAL ARTICLE

3D Culture System for Human Adrenal Glands That Uses a Sequential Processing Medium to Facilitate Cortical-Medullary Cell Development

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SUMMARY

Background: **The human adrenal gland is composed of the cortex and the medulla, which contain different function cells. The aim of this study was to build a 3D culture system for human adrenal glands.**
Methods: **Human fetal adrenal tissues were digested into a cell suspension culture and processed in three-phase 3D cultures.**
Results: **Apparent spheroids could be seen from the 4th day on. After 21 days of 3D culture, steroid synthesis cells were evident via CYP17A1+ immunohistochemical staining and flow cytometry analysis. Electron microscopy analysis showed that these cells were present in lipid droplets in the cytoplasm. Meanwhile, TH+ cells represented catecholamine-producing cells, and these cells exhibited electron density particle gathering in the cytoplasm. Dehydroepiandrosterone and epinephrine syntheses were further confirmed via enzyme-linked immunosorbent assay.**
Conclusions: **We established a 3D culture system for human adrenal glands by using a sequential processing medium to facilitate cortical–medullary cell development.**
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Supplementary Data

Table S1.

Target Name	Target type	Target Sequence
OCT4	Pluripotency marker	F: AAAGCTCTGCAGAAAGAACTCG
		R: GTCGTTTGGCTGAATACCTTCC
NANOG	Pluripotency marker	F: AGATGCCTCACACGGAGACTGTC
		R: TGGGTTGTTTGCCTTTGGGACTG
SOX10	NC lineage	F: TCTGGAGGCTGCTGAACGAA
		R: AAGTGGGCGCTCTTGTAGTG
SF-1	Steroidogenic factor 1	F: GAAGACCTGACTCGTAAACTGC
		R: CCTCGCTATTGTAGATGGGCT
b-catenin	WNT signaling marker	F: CATCTACACAGTTTGATGCTGCT
		R: GCAGTTTTGTCAGTTCAGGGA
PTCH1	Hedgehog ligands receptor	F: CACCATCCTCGGCGTTCTCAATG
		R: GTGTGGGCAGGCGGTTCAAG
HAND2	SCPs marker	F: ATGAGTCTGGTAGGTGGTTTTCC
		R: CATACTCGGGGCTGTAGGACA
GATA3	SCPs marker	F: TAACATCGACGGTCAAGGCAAC
		R: GTAGGGATCCATGAAGCAGAGG

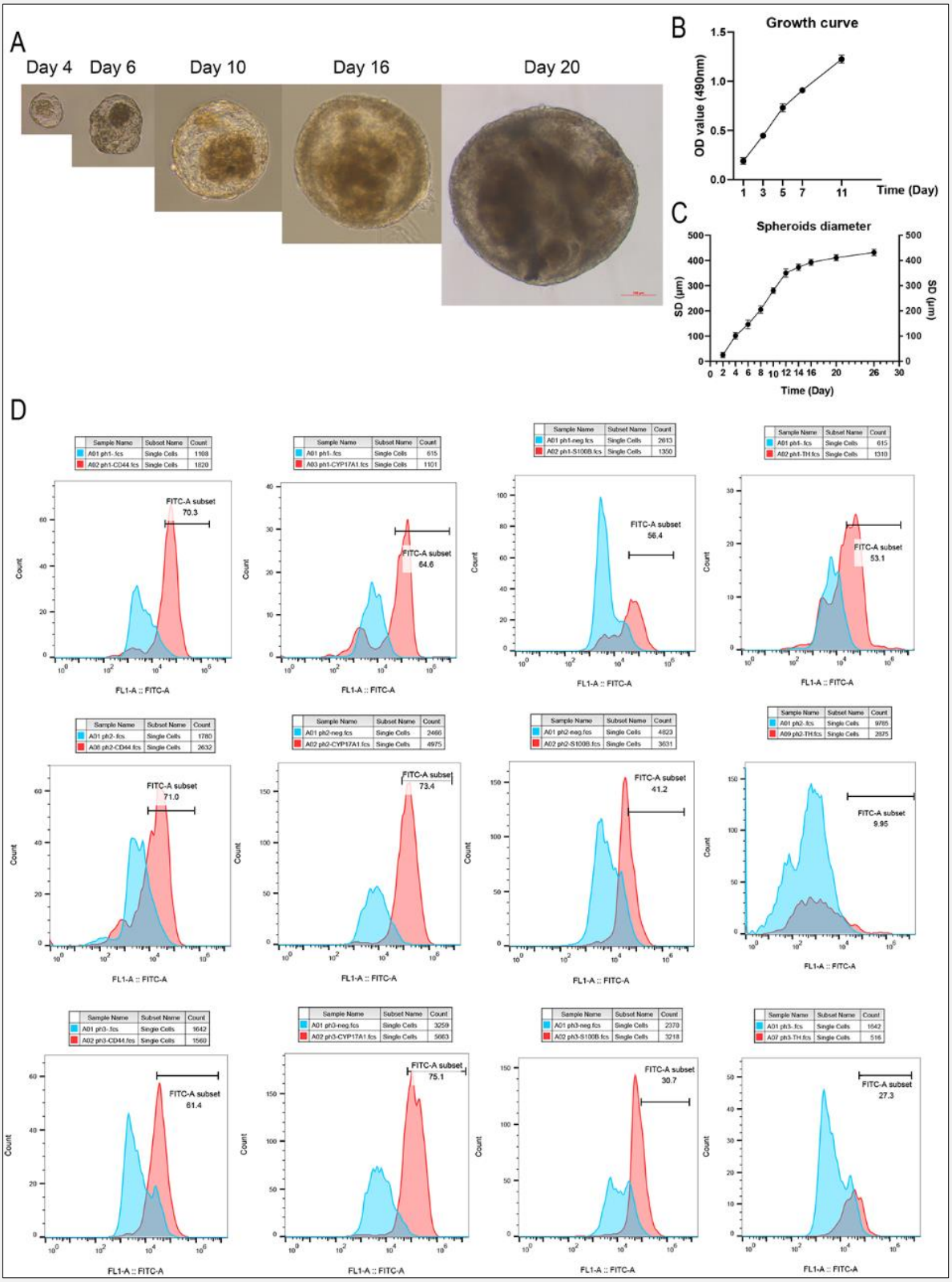

Figure 1S. Growth and function of adrenal organoids. (A) Growth of adrenal spheroids under light microscope. (B) Diameter of spheroids from 2 days to 26 days. (C) Flow cytometry analysis of CD44+, CYP17A1+, S100B+, and TH+ cells expressed at the different stages in the spheroids.