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Supplementary Materials

Strategies for Developing Functional Secretory Epithelia from Porcine Salivary Gland Explant Outgrowth Culture Models

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Table S1. List of primary antibodies (conjugated and unconjugated) used for flow cytometry or immunofluorescence imaging. Manufacturers location: Biolegend, San Diego, CA, USA; Abcam, Cambridge, UK; Cell Signaling Technology, Danvers, MA, USA; Novus Biologicals, Centennial, CO USA; R&D systems, Minneapolis, MN, USA; Santa Cruz, Dallas, TX, USA; Biorbyt, Cambridge, UK; Merck, Darmstadt, Germany.

Antibody	Dilution used	Catalog No.	Manufacturer
Alexa Fluor 488 anti-CD29	1:300	303015	Biolegend
Alexa Fluor 647 anti-CD44	1:200	103017	Biolegend
PE anti-CD90	1:80	328109	Biolegend
Alexa Fluor 647 anti-CD34	1:300	343507	Biolegend
Pacific Blue anti-CD45	1:200	304021	Biolegend
anti-KRT5	1:200	AB24647	Abcam
anti-KRT14	1:200	181595	Abcam
anti-E-cadherin	1:100	3195	Cell Signaling Technology
anti-Ki67	1:100	AF7617	Novus Biologicals
anti-αSMA	1:200	5694	Abcam
anti-beta-III tubulin	1:200	MAB1195	R&D systems
anti-Chrm3/M3	1:200	SC-9108	Santa Cruz
anti-Aquaporin 1	1:50	orb10122	Biorbyt
anti-Perlecan	1:500	MAB1948P	Merck

Table S2. Oligonucleotide forward and reverse primer sequences optimized for porcine SG tissues.

Gene	Forward sequence	Reverse sequence	NCBI reference
CD29	TGCAACCCCAACTACACTG	TTACAGACCCCACATTCACAG	NM_213968
CD90	TCCAGCAAGTACGACATCAAG	GAGAGGTGGAGTTCGCATG	NM_001146129
krt5	CGTAGTCAATCAAGCCTATT	GAAGCAGAGCGTAGAATC	XM_003126173
krt14	GAGATGTGACCTCCACTC	TAGTTCTTGGTGCGAAGG	XM_021067000
krt19	TCAGTGTGGAGGTTGATTCG	CTCATACTGGCTTCTCATGTCG	XM_003131437
kit	CGGAGAGCATTCAGACTTA	GCATCATCATCATCATATTGTTAC	NM_001044525
amy1a	AGCCCTTGTCTTTGTTGATA	ACTCGTGTGAATCCGTAAG	XM_021090124
aqp5	ATGATTCTGACCTTCCAGCTG	GTGAAGTAGATCCCCACAAGG	NM_001110424

Table S3. Expression of other mesenchymal and SG transcriptome markers in SMG-DC and PG-DC. mRNA expression from PCR is represented as fold change in cells from P1 relative to fresh SG tissue biopsies and normalized to a house keeping gene (S29). F.C.: fold change. SEM: standard error of the mean. * indicates $p < 0.05$ between SMG-DC passage 1 cells and fresh SMG tissue biopsies (baseline group). ** indicates $p < 0.05$ between PG-DC passage 1 cells and fresh PG tissue biopsies (baseline group).

SMG-DC		
Gene	**Mean F.C.**	**SEM**
CD29	52.27*	18.56
CD90	7.62*	2.90
krt5	1.25	0.20
kit	0.07	0.04
krt14	8.55*	1.39
krt19	0.80	1.03
amy1a	0.06*	0.01
aqp5	0.02*	0.01
PG-DC		
Gene	**Mean F.C.**	**SEM**
CD29	37.24**	7.17
CD90	10.31**	1.76
krt5	0.86**	0.08
kit	0.03	0.01
krt14	4.74**	0.21
krt19	2.65	0.09
amy1a	<0.01**	<0.001
aqp5	0.02**	0.01

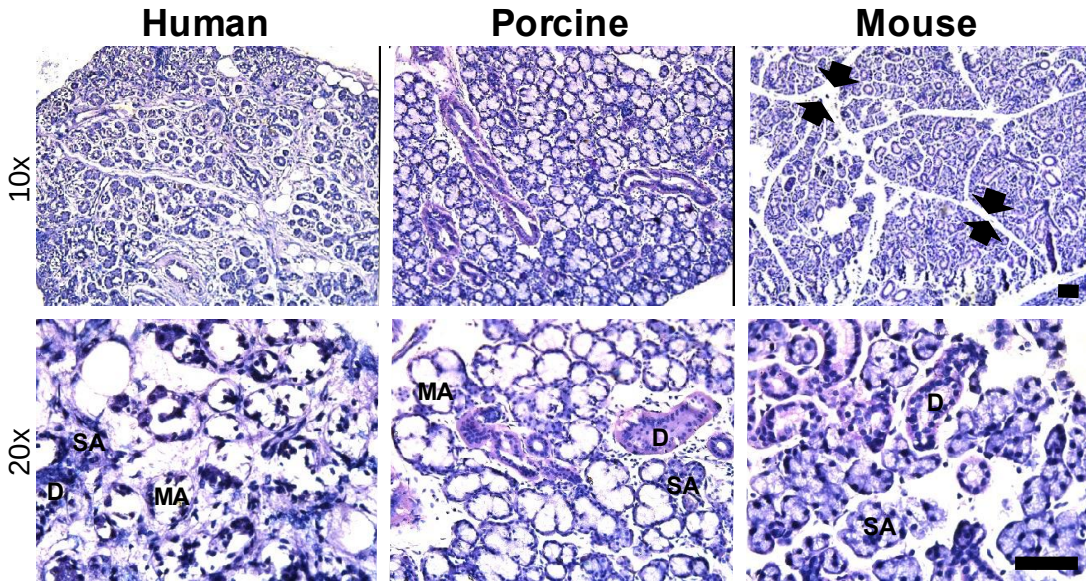

Figure S1. Human major salivary glands share very similar histological features and glandular sizes with the porcine glands, but not with the mouse submandibular glands. Mouse glands have a prominent connective tissue capsule (black arrows) surrounding all acinar lobes, and they lack mucous acinar cells. Hematoxylin and eosin staining. Scale bar: 100 μm. Legend - SA: serous acini. MA: mucous acini. D: Ducts.

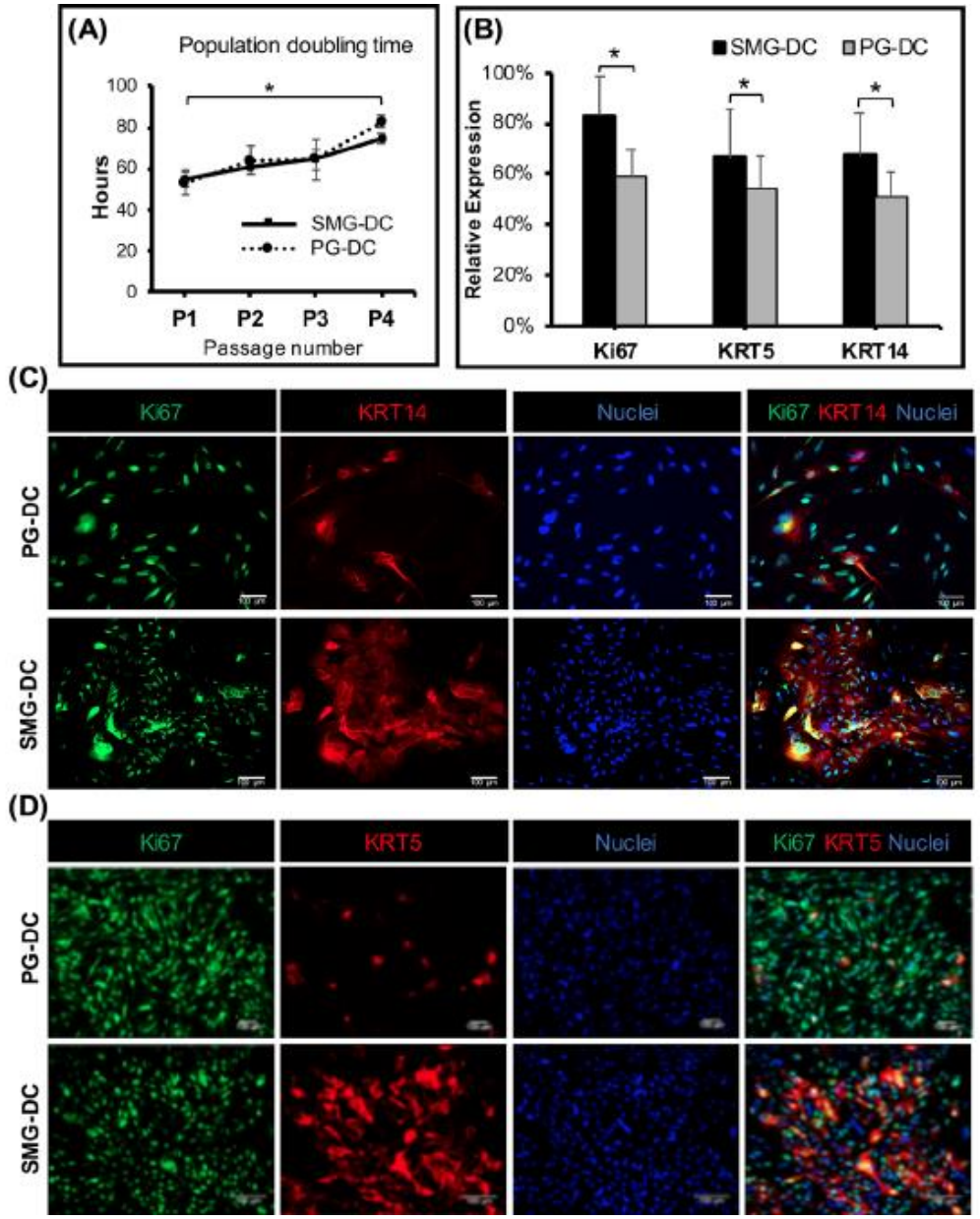

Figure S2. Cellular expansion through passaging and proliferation of epithelial progenitors in non-confluent undifferentiated SMG-DC and PG-DC cultures. **(A)** Cellular expansion was assessed by population doubling time (PDT) from passage 1 through 4 (P1–P4) of cell cultures. No significant difference was found between the first 3 passages ($N = 3–5$). *$p < 0.05$ when comparing passage 1 to 4. There were no differences in the PDT between SMG and PG through passage 1 to 4. **(B)** Quantification of Ki67+, KRT14+, KRT5+ cell subpopulations in SMG-DC and PG-DC. The y-axis represents protein expression relative to total nuclear/cell counts. *$p < 0.05$ when comparing SMG-DC with PG-DC. SMG-DC: submandibular gland-derived cells. **(C)** Abundant expression of pro-mitotic proliferative markers (Ki67) and epithelial stem/progenitor cells (KRT14) in SMG-DC and PG-DC after immunofluorescence staining at first subculture. Scale bar: 100 μm. **(D)** Expression of pro-mitotic proliferative markers (Ki67) and epithelial stem/progenitor cells (KRT5) in SMG-DC and PG-DC after immunofluorescence staining at first subculture. Scale bar: 100 μm. PG-DC: parotid gland-derived cells. SMG-DC: submandibular gland-derived cells.

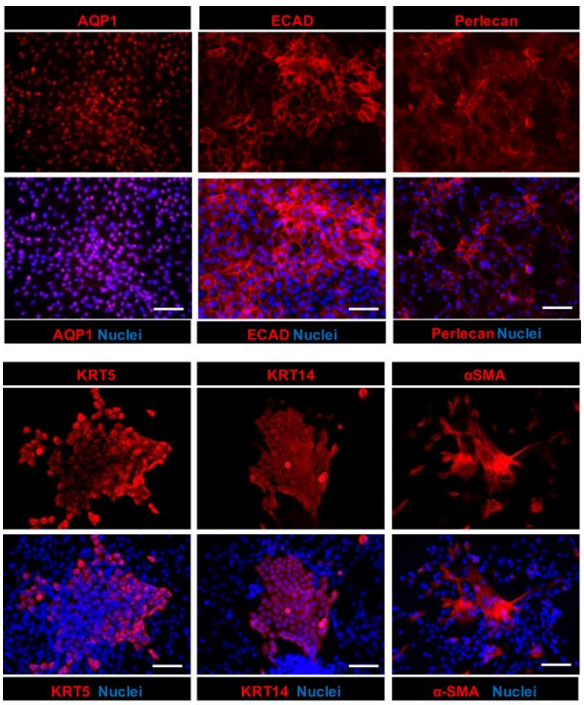

Figure S3. Expression of SG-specific acinar and ductal epithelial, myoepithelial and basement membrane markers after SG differentiation. Fluorescence microscopy images with z-stack maximum intensity projections are shown from immunohistochemistry for Aquaporin 1 (AQP1), E-cadherin (ECAD), KRT5, KRT14, Smooth muscle actin (αSMA) epithelial markers and counterstained with a nuclear dye. Perlecan was used to identify the basement membrane. Scale bar: 100 μm.

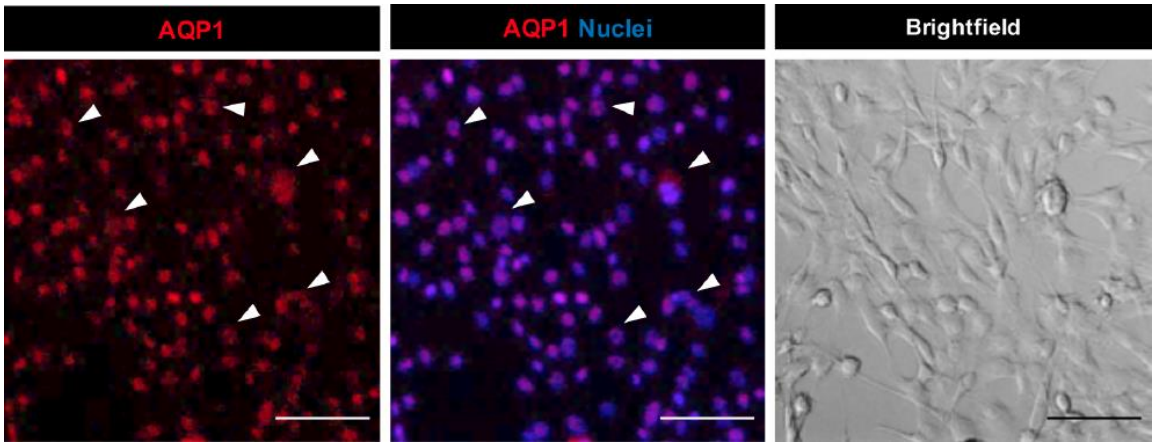

Figure S4. AQP1 water channels were not precisely located at the apical membrane after SG differentiation. Fluorescence microscopy images with z-stack maximum intensity projections are shown from immunohistochemistry for AQP1 at higher magnification, and counterstained with a nuclear dye. AQP1 appeared punctate around and overlapping the nuclei (white arrowheads) since this is a projection of multiple stacks. Scale bar: 100 μm.

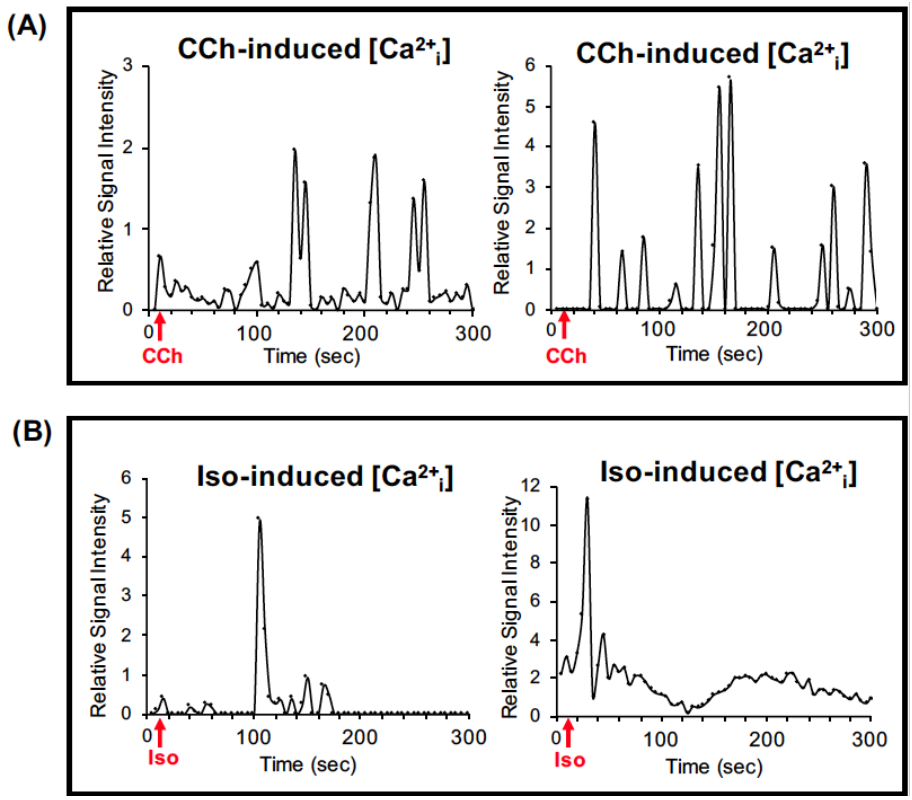

Figure S5. Intracellular Ca^{2+} influx tracking in differentiated SG cells upon cholinergic and adrenergic stimulation. (**A**,**B**) Intracellular calcium influx in differentiated SG cells after (**A**) cholinergic stimulation with Carbachol (CCh) and (**B**) adrenergic stimulation with Isoproterenol (Iso).