Effect of Pig-Adipose-Derived Stem Cells' Conditioned Media on Skin Wound-Healing Characteristics In Vitro

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Supplementary Table S1: Gene names and primer-probe sets used in real-time PCR.

Inventoried TaqMan® Gene Expression Assays			
Gene symbol	**Gene name**		**Primer and probe sets ID**
CCN2 (CTGF)	Cellular communication network factor 2 (Connective tissue growth factor)		Ss03392397_m1
COL1A1	Collagen type I alpha 1 chain		Ss03373340_m1
COL3A1	Collagen type III alpha 1 chain		Ss04323772_g1
FN1	Fibronectin 1		Ss03373673_m1
IVL	Involucrin		Ss03822369_s1
KRT1	Keratin 1		Ss03372954_m1
KRT6A	Keratin 6a		Ss06938590_s1
TGFB1	Transforming growth factor beta 1		Ss04955543_m1
TGFB3	Transforming growth factor beta 1		Ss03394351_m1
TNC	Tenascin C		Ss03394416_m1
HPRT1	Hypoxanthine phosphoribosyltransferase 1		Ss03388274
Made to order TaqMan® Gene Expression Assays			
Gene symbol	**Gene name**	**Primer/probe**	**Sequence 5′→3′**
AXIN2	Axin2	Forward primer Reverse primer Probe	GAGGGAGAAATGCGTGGATA GGTTTCAGCTGCTTGGAGAC ACTTCTGGTTTGCCTGCAAT
KRT10	Keratin 10	Forward primer	CCCAACTAGCCCTGAAACAA GTATTCGGCATTCTGGCACT

		Reverse primer Probe	CTACTGTGTGCAGCTCGCTC
KRT17	Keratin 17	Forward primer Reverse primer Probe	GAACCACGAGGAGGAGATGA CTCCGTCTTGCTGAAGAACC GTACGAGAAGATGGCCGAGA
WNT10A	Wingless family member 10A	Forward primer Reverse primer Probe	ACGAGTGCCAACACCAGTTC AGGCACTCTCTCGGAAACCT GCGCTGGAACTGCTCTAGTC
WNT11	Wingless family member 11	Forward primer Reverse primer Probe	GAGCTCATGCACACTGTCGT TCTCTCCAGGTCAAGCAGGT ACTGCTCCTCCATCGAGCT

Supplementary Table S2: Presentation of statistical analysis depicting the effect of different media (Ctrl medium, CM-Nor, or CM-Hyp) on the migration of pKERs over the time course (statistics refer to Figure 2A in the main body of manuscript).

Hours [h]	Group	lsmean	lower.CL	upper.CL	SE	df	8	24	48
0	**Ctrl medium**	100,0	83,1	116,9	7,96	15,4	0,94	0,935	**0,021**
8	**Ctrl medium**	95,2	78,3	112,1	7,96	15,4		0,656	**0,004**
24	**Ctrl medium**	105,0	88,0	121,9	7,96	15,4			0,084
48	**Ctrl medium**	125,5	108,6	142,5	7,96	15,4			
0	**CM-Nor**	100,0	83,1	116,9	7,96	15,4	0,954	0,927	0,991
8	**CM-Nor**	95,6	78,7	112,6	7,96	15,4		1,000	0,996
24	**CM-Nor**	94,8	77,9	111,7	7,96	15,4			0,988
48	**CM-Nor**	97,5	80,6	114,5	7,96	15,4			
0	**CM-Hyp**	100,0	83,1	116,9	7,96	15,4	0,228	**0,043**	0,133
8	**CM-Hyp**	83,7	66,7	100,6	7,96	15,4		0,856	0,992
24	**CM-Hyp**	77,0	60,0	93,9	7,96	15,4			0,956
48	**CM-Hyp**	81,3	64,3	98,2	7,96	15,4			

Supplementary Table S3: Presentation of statistical analysis depicting the effect of CM-Nor and CM-Nor +IL-1β on pKERs migration over the time course (statistics refer to Figure 2B in the main body of manuscript).

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	100,000	5,47	18,279	**<0,001**
Group CM-Nor+IL-1β	0,000	7,74	0,000	1,000
Hours 8	-4,374	7,74	-0,565	0,576
Hours 24	-5,185	7,74	-0,670	0,508
Hours 8	-2,466	7,74	-0,319	0,752
Group CM-Nor+IL-1β: Hours 8	0,032	10,94	0,003	0,998
Group CM-Nor+IL-1β: Hours 24	-0,549	10,94	-0,050	0,960
Group CM-Nor+IL-1β: Hours 48	17,557	10,94	1,605	0,118
Observations	**Residual Std. Error**	**R^2**	**Adjusted R^2**	
40	12,2	0,254	0,09	

Supplementary Table S4: Presentation of statistical analysis depicting the effect of CM-Hyp and CM-Hyp +IL-1β on pKERs migration over the time course (statistics refer to Figure 2C in the main body of manuscript).

Hours [h]	Group	lsmean	lower.CL	upper.CL	SE	df	8	24	48
0	**CM-Hyp**	100,0	82,1	117,9	8,54	19,4	0,384	0,126	0,268
8	**CM-Hyp**	83,7	65,8	101,5	8,54	19,4		0,909	0,995
24	**CM-Hyp**	77,0	59,1	94,8	8,54	19,4			0,973
48	**CM-Hyp**	81,3	63,4	99,1	8,54	19,4			
0	**CM-Hyp +IL-1β**	100,0	82,1	117,9	8,54	19,4	0,211	0,377	0,998
8	**CM-Hyp + IL-1β**	79,8	62,0	97,7	8,54	19,4		0,982	0,151
24	**CM-Hyp + IL-1β**	83,5	65,7	101,4	8,54	19,4			0,286
48	**CM-Hyp + IL-1β**	101,9	84,0	119,7	8,54	19,4			

Supplementary Table S5: Presentation of statistical analysis depicting the effect of different media (Ctrl medium, CM-Nor, or CM-Hyp) on pDFs migration over the time course (statistics refer to Figure 3A in the main body of manuscript).

Hours [h]	Group	lsmean	lower.CL	upper.CL	SE	df	24	48	72
0	**Ctrl medium**	100,00	93,08	106,92	3,35	23,9	**<0,001**	**<0,001**	**<0,001**
24	**Ctrl medium**	9,47	2,55	16,39	3,35	23,9		0,271	0,093
48	**Ctrl medium**	2,20	-4,72	9,12	3,35	23,9			0,944
72	**Ctrl medium**	0,00	-6,92	6,92	3,35	23,9			
0	**CM-Nor**	100,00	93,08	106,92	3,35	23,9	**<0,001**	**<0,001**	**<0,001**
24	**CM-Nor**	25,31	18,39	32,23	3,35	23,9		**<0,001**	**<0,001**
48	**CM-Nor**	4,58	-2,34	11,50	3,35	23,9			0,656
72	**CM-Nor**	0,00	-6,92	6,92	3,35	23,9			
0	**CM-Hyp**	100,00	93,08	106,92	3,35	23,9	**<0,001**	**<0,001**	**<0,001**
24	**CM-Hyp**	34,23	27,31	41,15	3,35	23,9		**<0,001**	**<0,001**
48	**CM-Hyp**	15,41	8,49	22,33	3,35	23,9			**0,002**
72	**CM-Hyp**	0,00	-6,92	6,92	3,35	23,9			

Supplementary Table S6: Presentation of statistical analysis depicting the effect of CM-Nor and CM-Nor +bFGF on pDFs migration over the time course (statistics refer to Figure 3B in the main body of manuscript).

Hours [h]	Group	lsmean	lower.CL	upper.CL	SE	df	24	48	72
0	**CM-Nor**	100,00	92,96	107,04	3,46	32	**<0,001**	**<0,001**	**<0,001**
24	**CM-Nor**	25,31	18,27	32,35	3,46	32		**<0,001**	**<0,001**
48	**CM-Nor**	4,58	-2,46	11,62	3,46	32			0,785
72	**CM-Nor**	0,00	-7,04	7,04	3,46	32			
0	**CM-Nor+bFGF**	100,00	92,96	107,04	3,46	32	**<0,001**	**<0,001**	**<0,001**
24	**CM-Nor+bFGF**	18,89	11,85	25,94	3,46	32		**0,003**	**0,003**
48	**CM-Nor+bFGF**	0,00	-7,04	7,04	3,46	32			1,000
72	**CM-Nor+bFGF**	0,00	-7,04	7,04	3,46	32			

Supplementary Table S7: Presentation of statistical analysis depicting the effect of CM-Hyp and CM-Hyp +bFGF on pDFs migration over the time course (statistics refer to Figure 3C in the main body of manuscript).

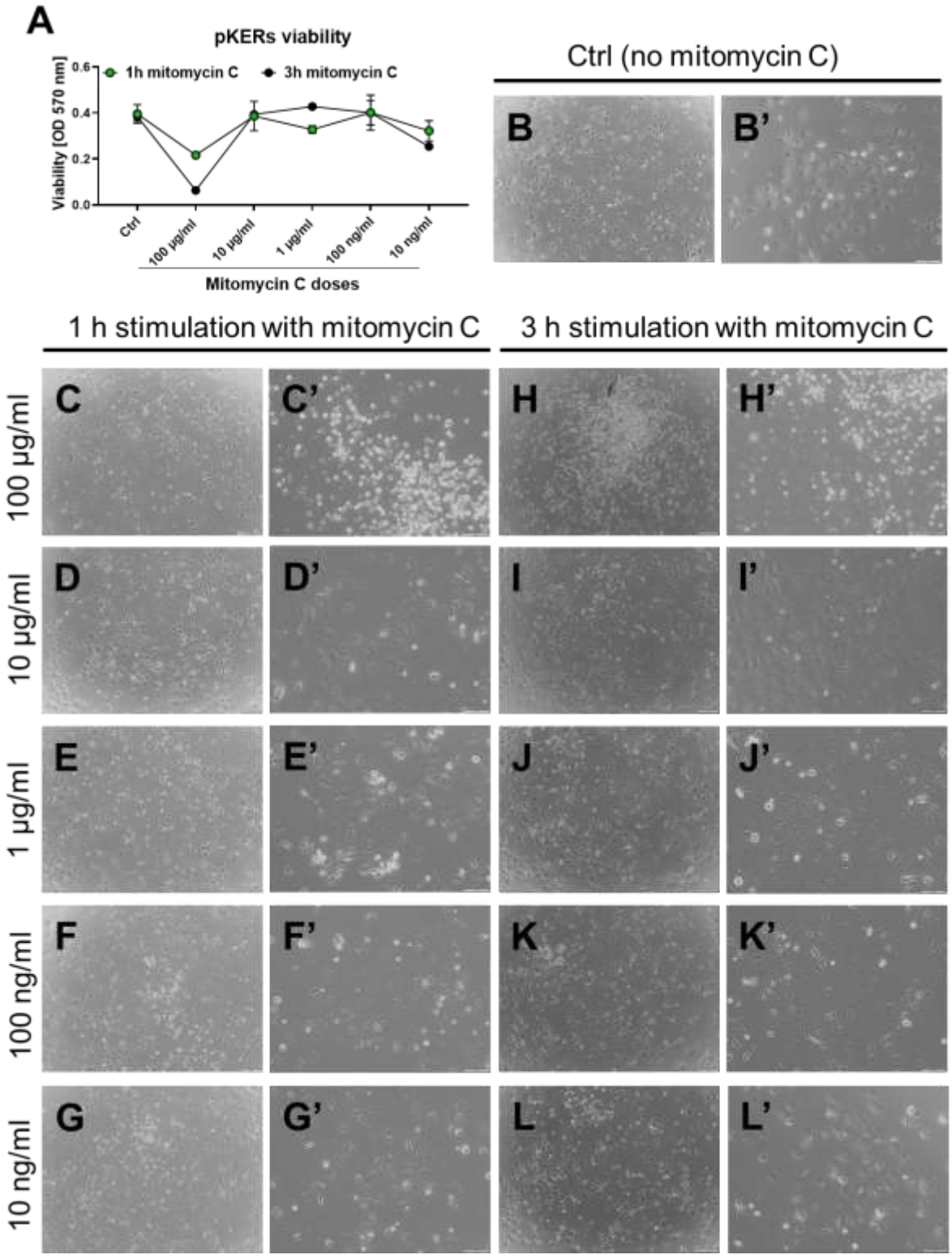
Hours [h]	Group	lsmean	lower.CL	upper.CL	SE	df	24	48	72
0	**CM-Hyp**	100,0	93,70	106,30	3,09	32	**<0,001**	**<0,001**	**<0,001**
24	**CM-Hyp**	34,2	27,93	40,52	3,09	32		**0,001**	**<0,001**
48	**CM-Hyp**	15,4	9,11	21,70	3,09	32			**0,008**
72	**CM-Hyp**	0,0	-6,29	6,29	3,09	32			
0	**CM-Hyp+bFGF**	100,0	93,70	106,30	3,09	32	**<0,001**	**<0,001**	**<0,001**
24	**CM-Hyp+bFGF**	17,7	11,43	24,02	3,09	32		**0,002**	**0,002**
48	**CM-Hyp+bFGF**	0,0	-6,29	6,29	3,09	32			1,000
72	**CM-Hyp+bFGF**	0,0	-6,29	6,29	3,09	32			

Supplementary Table S8: Presentation of statistical analysis depicting the effect of different media (Ctrl medium, CM-Nor, or CM-Hyp) on pDFs contractility over the time course (statistics refer to Figure 4A in the main body of manuscript).

Day	Group	lsmean	lower.CL	upper.CL	SE	df	1	2	3	4	5	6	7	8	9
0	**Ctrl medium**	100,0	93,3	106,7	3,23	21,2	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**
1	**Ctrl medium**	84,1	77,4	90,8	3,23	21,2		0,289	0,106	0,2	**0,036**	**<0,001**	**<0,001**	**<0,001**	**<0,001**
2	**Ctrl medium**	75,3	68,5	82,0	3,23	21,2			1,000	1,000	0,997	0,453	0,252	0,325	0,205
3	**Ctrl medium**	73,6	66,9	80,4	3,23	21,2				1,000	1,000	0,746	0,517	0,612	0,448
4	**Ctrl medium**	74,6	67,9	81,3	3,23	21,2					1,000	0,573	0,347	0,433	0,289
5	**Ctrl medium**	72,2	65,5	78,9	3,23	21,2						0,924	0,768	0,842	0,705
6	**Ctrl medium**	66,9	59,8	74,0	3,48	27,2							1,000	1,000	1,000

7	**Ctrl medium**	65,6	58,5	72,7	3,48	27,2								1,000	1,000
8	**Ctrl medium**	66,1	59,0	73,3	3,48	27,2									1,000
9	**Ctrl medium**	65,2	58,1	72,4	3,48	27,2									
0	**CM-Nor**	100,0	93,3	106,7	3,23	21,2	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**
1	**CM-Nor**	75,8	69,1	82,5	3,23	21,2		0,625	0,211	0,104	**0,004**	**0,001**	**<0,001**	**<0,001**	**<0,001**
2	**CM-Nor**	68,8	62,1	75,5	3,23	21,2			1,000	0,992	0,531	0,254	0,142	**0,002**	**<0,001**
3	**CM-Nor**	66,4	59,6	73,1	3,23	21,2				1,000	0,918	0,66	0,473	**0,02**	**0,003**
4	**CM-Nor**	65,3	58,5	72,0	3,23	21,2					0,982	0,834	0,672	**0,046**	**0,009**
5	**CM-Nor**	61,3	54,6	68,0	3,23	21,2						1,000	0,998	0,452	0,163
6	**CM-Nor**	59,1	52,0	66,3	3,48	27,2							1,000	0,863	0,544
7	**CM-Nor**	58,1	51,0	65,3	3,48	27,2								0,951	0,72
8	**CM-Nor**	52,9	45,8	60,1	3,48	27,2									1,000
9	**CM-Nor**	50,9	43,7	58,0	3,48	27,2									
0	**CM-Hyp**	100,0	93,3	106,7	3,23	21,2	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**	**<0,001**
1	**CM-Hyp**	72,7	65,9	79,4	3,23	21,2		0,504	0,078	**0,028**	**0,04**	**0,012**	**0,007**	**<0,001**	**<0,001**
2	**CM-Hyp**	65,1	58,3	71,8	3,23	21,2			0,995	0,952	0,975	0,793	0,71	0,236	**0,003**
3	**CM-Hyp**	61,8	55,0	68,5	3,23	21,2				1,000	1,000	0,998	0,994	0,783	0,052
4	**CM-Hyp**	60,4	53,7	67,2	3,23	21,2					1,000	1,000	1,000	0,934	0,127
5	**CM-Hyp**	60,9	54,2	67,6	3,23	21,2						1,000	0,999	0,895	0,096
6	**CM-Hyp**	58,6	51,5	65,8	3,48	27,2							1,000	0,998	0,413
7	**CM-Hyp**	58,1	51,0	65,2	3,48	27,2								0,999	0,5
8	**CM-Hyp**	55,3	48,1	62,4	3,48	27,2									0,915
9	**CM-Hyp**	49,6	42,4	56,7	3,48	27,2									

Supplementary Figure S1



Supplementary Figure S1.
Dose and time response of pKERs (p = 1) to mitomycin C stimulation.

Cells were seeded onto 24-well plates at a density of 0.5 × 10^6 per well and allow to growth in keratinocyte culture medium (EpiLife™; Gibco, Life Technologies Corporation, Grand Island, NY, USA). After 48 h, the cells were treated with mitomycin C (Sigma-Aldrich Co., St. Louis, MO, USA) at the concentrations of 100 μg/mL, 10 μg/mL, 1 μg/mL, 100 ng/mL, or 10 ng/mL in keratinocyte culture medium for 3 h or 1 h, cultures with no mitomycin C were used as control. Next, the treatment was removed, cells were washed with PBS and fresh keratinocyte culture medium was added. After 24 h toxicity of mitomycin C was examined via cell viability assay based on MTT (3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyltetrazolium bromide; Sigma-Aldrich Co., St. Louis, MO, USA) colorimetric method [1].
There were no differences in cell viability between control cells (Ctrl; A, B, B′) and pKERs pre-treated with mitomycin C at doses 10 μg/ml, 1 μg/ml, 100 ng/ml, or 10 ng/ml, regardless of the exposure time (1 h or 3 h; A, D – L′). However, higher dose of mitomycicn C (100 μg/ml) showed toxic effect on pKERs manifested by the decrease in their viability and their massive detachment from the growth surface (A, C, C′, H, H′). Mitomycin C at dose of 10 μg/ml that was used in our and other studies [1-5] had no effect on pKERs viability as compared to control-treated cells (A, B, B′, D, D′, I, I′) and relative to lower doses of the treatment (A, E-L′). These results indicate that the concentration of 10 μg/ml mitomycin C is the highest effective dose that subsequently has no toxic effect on the cell viability. Moreover, no differences between both tested time points (1 h or 3 h) were observed for mitomycin C at doses 10 μg/ml, 1 μg/ml, 100 ng/ml, or 10 ng/ml (A, B, B′, D-L′). However, exposure to dose 100 μg/ml for 3 h led to greater decrease in cell viability as compared to the effect observed after 1 h of administration with the treatment (A, C-H′). Therefore, to minimize the negative effect of mitomycin C pKERs were pre-treated for 1 h. Together, these comparative data indicate that pre-tretment with mitomycin C at the concentration of 10 μg/ml for 1 h has no toxic effect on pKERs and the condition does not contribute to the cell detachment from the growth surface in wound migration assay.

Figure S1 abbreviations: pKERs; pig keratinocytes. Scale bars: 200 μm (B-L); 100 μm (B′-L′).

References:

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