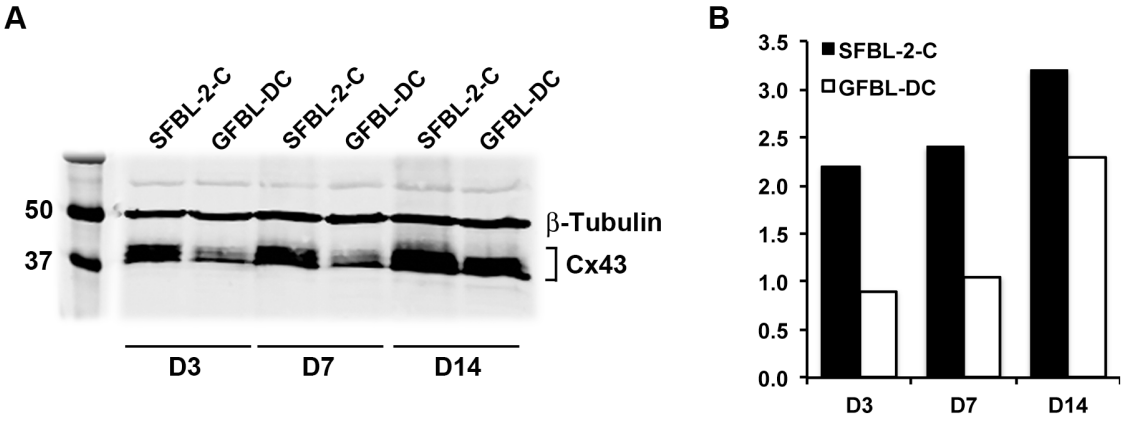
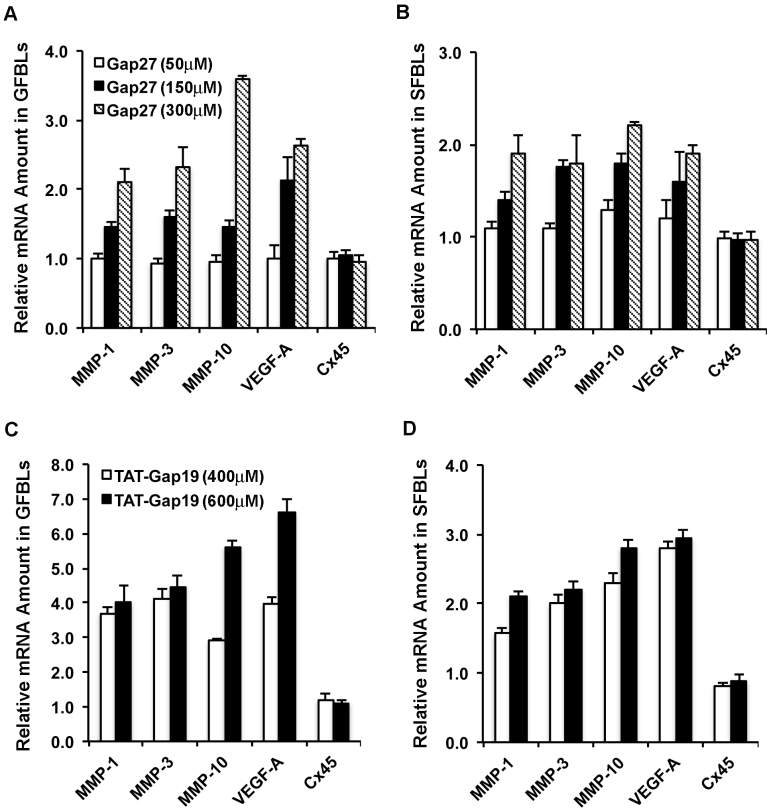
Supplemental Files

Supplemental Figure S1. **Western blotting analysis of Cx43 in three-dimensional cultures of skin and gingival fibroblasts over time.** Western blotting analysis (A) and quantitation (B) of Cx43 in 3D cultures of skin (SFBL-2-C) and gingival fibroblasts (GFBL-DC), 3-, 7-, and 14-days post-seeding. The abundance of Cx43 is increased over time in both cell types. Sample loading was normalized for β-Tubulin levels. Results from one experiment are shown.



Supplemental Figure S2. The expression of a set of genes in human gingival and skin fibroblasts treated with increasing concentrations of Gap27 or TAT-Gap19 relative to control samples. Day-7 3D cultures of GFBLs (GFBL-DC) (A and C) and SFBLs (SFBL-1-2) (B and D) were serum-starved for 24 h and then treated with (A and B) increasing concentrations of Gap27 (50, 150, and 300 μM) or control peptide (50, 150, and 300 μM), or (C and D) increasing concentrations of TAT-Gap19 (400 and 600 μM) or control peptide (400 and 600 μM) for 24 h, and expression of a set of genes involved in wound healing was analyzed by qPCR. Results represent mean ± s.e.m. of mRNA amount relative to control peptide-treated cells from three parallel samples from one experiment. VEGF-A: Vascular Endothelial Growth Factor-A.



Supplemental Table S1. List of the human gingival and skin fibroblast lines used for the study.

Cell line name	Origin	Sex	Age (years)
GFBL-DC	Attached gingiva	Male	41
GFBL-OL	Attached gingiva	Male	30
GFBL-HN	Attached gingiva	Female	18
GFBL-DW	Attached gingiva	Female	30
GFBL-IE	Attached gingiva	Male	26
SFBL-2-C	Caucasian breast dermis	Female	40
SFBL-1-2	Caucasian breast dermis	Female	44
SFBL-4-1	Caucasian breast dermis	Female	41
SFBL-302	Caucasian breast dermis	Female	38
SFBL-406	Caucasian breast dermis	Female	35

Supplemental Table S2. List of antibodies used for immunostaining and Western blotting.

Antibody	**Manufacturer**	**Source**	**Dilution**	
			Immunostaining	**Western blotting**
Anti-Vimentin (M7020; Vim 3B4)	DakoCytomation, Burlington, ON, CA, USA	Mouse	1:200	
Anti-Connexin 43 (C6219)	Sigma-Aldrich, St. Louis, MO, USA	Rabbit	1:800	1:8000
Cx43(E2)	Kindly provided by Dr. Jean X. Jiang, University of Texas Health Science Center, San Antonio, TX, USA	Rabbit	1:300	
Anti-β-Tubulin (ab21057)	Abcam Inc., Eugene, OR, USA	Goat		1:1000

Supplemental Table S3. Primers used for quantitative real-time RT-PCR.

GeneBank	Gene	Primer sequence	Orientation	Location	Amplicon (bp)
MMPs and TIMPs					
NM_002421	MMP-1	GCTAACAAATACTGGAGGTATGATG	Forward	1250-1275	100
		GTCATGTGCTATCATTTTGGGA	Reverse	1304-1325	
NM_001166308	MMP-3	ATGATGAACAATGGACAAAGGA	Forward	661-682	91
		GAGTGAAAGAGACCCAGGGA	Reverse	751-732	
NM_002425	MMP-10	TTATACACCAGATTTGCCAAGA	Forward	394-415	56
		TTCAGAGCTTTCTCAATGG	Reverse	450-432	
NM_004995	MMP-14	TCTCCCAGAGGGTCATTCAT	Forward	618-1637	70
		TTCCAGTATTTGTTCCCCTTGTAG	Reverse	1688-1665	
NM_003254	TIMP-1	CTGTGTCCCACCCCACC	Forward	267-283	64
		GAACTTGGCCCTGATGACGA	Reverse	330-311	
NM_003255	TIMP-2	ACATTTATGGCAACCCTATCAA	Forward	481-502	70
		TCAGGCCCTTTGAACATCTTTA	Reverse	550-529	
NM_000362	TIMP-3	AGGACGCCTTCTGCAAC	Forward	1281-1297	68
		CTCCTTTACCAGCTTCTTCC	Reverse	1348-1329	
NM_003256	TIMP-4	ACCTGTCCTTGGTGCAGA	Forward	927-944	80
		TGTAGCAGGTGGTGATTTGG	Reverse	1004-985	
Fibrillar ECM proteins					
BC036531	Collagen type I (alpha 1)	AACCAAGGCTGCAACCTGGA	Forward	3951-3970	80
		GGCTGAGTAGGGTACACGCAGG	Reverse	4030-4009	
NM_000090	Collagen type III (alpha 1)	CTCCTGGGATTAATGGTAGT	Forward	1271-1290	70
		CCAGGAGCTCCAGGAAT	Reverse	1340-1324	
NM_212482	EDA-FN (Extra Domain A-Fibronectin)	CACAGTCAGTGTGGTTGCCT	Forward	5633-5652	68
		CTGTGGACTGGGTTCCAATCA	Reverse	5700-5680	
NM_212482	EDB-FN (Extra Domain B-Fibronectin)	CAGTAGTTGCGGCAGGAGAA	Forward	4168-4188	65
		GTATCCTACTGAGGAGTCCACAAAATC	Reverse	4232-4206	
Matricellular proteins					
NM_002160	TN-C (Tenascin-C)	CAACCTGATGGGGAGATATGGGGA	Forward	6769-6792	75

		GAGTGTTCGTGGCCCTTCCAG	Reverse	6846-6826	
Contractility and myofibroblast associated proteins					
NM_001613	α –SMA (α-Smooth Muscle Actin)	AGCGTGGCTATTCCTTCGT	Forward	637-655	97
		CTCATTTTCAAAGTCCAGAGCTACA	Reverse	733-707	
NM_005964	NMMIIB (Non-Muscle Myosin IIB)	CCGTTTTACATAATCTGAAGGATC	Forward	395-418	98
		TTGGAAGATTCTTGTAAGGGTT	Reverse	493-472	
Small leucine-rich proteoglycans					
BT019800	DCN (Decorin)	CTGACACAACTCTGCTAGAC	Forward	242-261	97
		GACAAGAATCAATGCGTGAAG	Reverse	339-319	
NM_002023	FMOD (Fibromodulin)	CACAATGAGATCCAGGAAG	Forward	761-779	85
		TCCGAAGGTGGTTATAACTC	Reverse	845-826	
TGF-β signaling related genes					
NM_000660	TGF-β1	CAACGAAATCTATGACAAGTTCAAGCAG	Forward	1218-1245	76
		CTTCTCGGAGCTCTGATGTG	Reverse	1294-1275	
NM_003239	TGF-β3	ACACCAATTACTGCTTCCGCAA	Forward	1161-1182	81
		GCCTAGATCCTGTCGGAAGTC	Reverse	1242-1220	
NM_005966	NAB1 (NGFI-A Binding Protein-1)	CAAAGTCCCACTCATCAGAGA	Forward	1930-1950	114
		TCACAGCTATCTTGAATCTTCAG	Reverse	2043-2020	
Growth factors and cytokines					
NM_001171630	VEGF-A (Vascular Endothelial Growth Factor-A)	AGTGTGTGCCCACTGAGGA	Forward	1316-1334	97
		GTGCTGTAGGAAGCTCATCTC	Reverse	1413-1393	
NM_199168	CXCL12/SDF-1α	TACAGATGCCCATGCCGA	Forward	174-191	93
		CTGAAGGGCACAGTTTGGAG	Reverse	266-247	
Cell-cell junction proteins					
NM_000165	Cx43	AGCAGTCTGCCTTTCGTTGTA	Forward	393-412	73
		GATTGGGAAAGACTTGTCATAGCAG	Reverse	466-442	
NM_001097519	Cx45	AGCTGGGTCCAACAAAAGC	Forward	1151-1169	108
		ACCATAAACTATGAGAAGCACAGATT	Reverse	1258-1233	
NM_001792	Cadherin-2	TGAGGAGTCAGTGAAGGAG	Forward	843-861	91

		CTTCTGCCTTTGTAGGTGG	Reverse	933-915	
Human and pig reference genes					
NM_002046	GAPDH (Glyceraldehydes-3-phosphate d-dehydrogenase)	CTTTGTCAAGCTCATTTCCTGGTA	Forward	1020-1043	70
		GGCCATGAGGTCCACCA	Reverse	1089-1073	
M31642	HPRT1 (Hypoxanthine phosphoribosyltransferase I)	TGTTGGATTTGAAATTCCAGACAAG	Forward	619-643	107
		CTTTTCCAGTTTCACTAATGACACAA	Reverse	727-700	
Human reference genes					
NM_021009	UBC (Ubiquitin C)	GTGGCACAGCTAGTTCCGT	Forward	371-389	96
		CTTCACGAAGATCTGCATTGTCA	Reverse	444-467	
NM_004048	B2M (Beta-2-microglobulin)	TGTCTTTCAGCAAGGACTGGTCTTTC	Forward	281-306	92
		ATGGTTCACACGGCAGGCATA	Reverse	351-372	
NM_001172085	TBP (TATAA-box binding protein)	TTCGGAGAGTTCTGGGATTG	Forward	542-562	94
Pig reference genes					
XM_001925271	UBC (Ubiquitin C)	TGCCGCTATACAATGCAG	Forward	161-178	90
		GACATTCTCAATGGTGTCA	Reverse	250-232	
AF452448	B2M (Beta-2-microglobulin)	TGTCTTTCAGCAAGGACTGGTCTTTC	Forward	3935-3960	90
		TGCTTCACGCGGCAGCTATAC	Reverse	4025-4005	

Supplemental Table S4. Regulation of the Expression of Wound Healing-Associated Genes by Cx43 GJ and HC Blocking Peptides in Gingival Fibroblasts in two- and three-Dimensional Cell Culture Models. Table compares the gene expression changes between day-7 3D (from present study) and traditional day-3 2D [Tarzemany et al., 2017] GFBL cultures treated with Gap27 (150 μM), TAT-Gap19 (400 μM) or corresponding control peptides for 24 h. Genes are indicated based on whether their expression was significantly ($p<0.05$; two-tailed Student's *t*-test) changed only by Gap27, only by TAT-Gap19, by both Gap27 and TAT-Gap19, or genes that were not regulated by Gap27 and TAT-Gap19 relative to corresponding controls. Results are from qPCR analysis of mRNA amount relative to control peptide-treated samples. Genes regulated by different mechanisms in 2D and 3D cultures are bolded. Results from 3D cultures (this study) represent three parallel GFBL strains (GFBL-DC, GFBL-IE, and GFBL-DW). Results from 2D cultures [Tarzemany et al., 2017] represent GFBL-DC from a minimum of three repeated experiments. Coll I: Collagen type I; Coll III: Collagen type III; EDA-FN: Extra Domain A-Fibronectin; EDB-FN: Extra Domain B-Fibronectin; TN-C: Tenascin-C; α-SMA: α-Smooth Muscle Actin; NMMIIB: Non-Muscle Myosin IIB, DCN: Decorin; FMOD: Fibromodulin; NAB1: NGFI-A Binding Protein-1; VEGF-A: Vascular Endothelial Growth Factor-A.

Genes not regulated by Gap27 and TAT-Gap19		**Genes regulated by Gap27 only**		**Genes regulated by TAT-Gap19 only**		**Genes regulated by both Gap27- and TAT-Gap19**	
2D	3D	2D	3D	2D	3D	2D	3D
TIMP-2 EDA-FN EDB-FN Cx45	TIMP-2 EDA-FN EDB-FN Cx45 **MMP-14** **α-SMA** **DCN** **FMOD**	TIMP-4 **TIMP-1** **TIMP-3** **FMOD** **Cadherin-2**	TIMP-4 **Coll III**		**TIMP-3** **Coll I** **NMMIIB** **NAB1** **CXCL12**	MMP-1 MMP-3 MMP-10 TGF-β1 TGF-β3 TN-C VEGF-A Cx43 **MMP-14** **Coll I** **Coll III** **α-SMA** **NMMIIB** **DCN** **NAB1** **CXCL12**	MMP-1 MMP-3 MMP-10 TGF-β1 TGF-β3 TN-C VEGF-A Cx43 **TIMP-1** **Cadherin-2**