Mechanical stress potentiates the differentiation of periodontal ligament stem cells into keratocytes

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Supplementary materials and methods

Isolation and characterization of PDLSCs

Human PDLSCs were isolated from surgically removed impacted third molars. The periodontal ligaments were cut into pieces (3-4 mm^3), and enzymatically digested in 3 mg/ml collagenase type I (Worthington Biochemicals Corp., Freehold, NJ) and 4 mg/ml dispase II (Roche Diagnostics, Indianapolis, IN) for 1 hour at 37°C. Single cell suspensions were obtained by passing cells through a 70 μm strainer (BD Falcon™ Labware, Franklin Lakes, NJ). The cells were seeded on plates and cultured in growing medium, Minimum Essential Medium-α (Life technologies, Grand Island, NY) with 10% FBS (Life technologies) and 1% penicillin-streptomycin (Life technologies). The PDLSCs used in the present project have recently been characterized in another study of ours (Chen et al.; *Stem Cell Res Ther* 2017). They were found to have clonogenic property, multi-lineage differentiation potential and positively expressed MSCs surface markers (CD73, CD90 and CD105), and negative expression for CD11b, CD19, CD34, CD45 and HLA-DR.

Western blot analysis

Protein was extracted from cell samples by RIPA lysis and centrifugation. The concentration was quantified using Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA). Loaded protein samples were ran for 1 hour at 150 V in pre-cast polyacrylamide gel (Bio-Rad), and subsequently transferred to a PVDF Blotting Membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for 1 hour at 100 V. After blocking in 5 % bovine serum albumin (BSA) in TBST for 1 hour, the blots were incubated with primary antibodies (Supplementary Table 3) overnight at 4 °C. After that, the blots were washed with TBST for three times, and then incubated with a HRP-linked secondary antibody (Supplementary Table 3) for 1 hour. After wash in TBST for another three times, the blots were incubated with ECL (GE Healthcare) for 5 minutes. Odyssey® Fc Dual-Mode Imaging System (LI-COR, Lincoln, NE, USA) was used to visualize the bands. And ImageJ analysis software (NIH) was used to evaluate the densitometry.

Supplementary Table 1. Probes used for qPCR.

Gene name	Gene symbol	Assay ID
Aldehyde dehydrogenase 3A1	*ALDH3A1*	Hs00964880_m1
CD34	*CD34*	Hs00990732_m1
Lumican	*LUM*	Hs00929860_m1
Collagen type I	*COL I*	Hs00164004_m1
Collagen type V	*COL V*	Hs00609133_m1
Alpha 2 smooth muscle actin	*ACTA2*	Hs00909449_m1
β-actin	*ACTB*	4352667

Supplementary Table 2. Primers used for qPCR.

Genes	5'-3'	Primers
Glyceraldehyde 3-phosphate	Forward	TGACGCTGGGGCTGGCATTG
dehydrogenase (*GAPDH*)	Reverse	GGCTGGTGGTCCAGGGGTCT
Scleraxis (*SCX*)	Forward	CGAGAACACCCAGCCCAAAC
	Reverse	CTCCGAATCGCAGTCTTTCTGTC
Tenomodulin (*TNMD*)	Forward	TGGGTGGTCCCTCAAGTGAAAGT
	Reverse	CTCGACGGCAGTAAATACAACAATA
Integrin alpha 1 (*ITGA1*)	Forward	AAGGGGAGAACTTCGGAGTGA
	Reverse	AAATGAGCAGCATTAACAGCAAC
Integrin alpha 2 (*ITGA2*)	Forward	GGGAATCAGTATTACACAACGGG
	Reverse	CCACAACATCTATGAGGGAAGGG
Integrin beta 1 (*ITGB1*)	Forward	TTCAGTGAATGGGAACAACGA
	Reverse	ATGCAAGGCCAATAAGAACAA
Non-muscle myosin II B (*MYH10*)	Forward	GCCGCCAACAAATTAGTCCGT
	Reverse	GCGTTTAAGCTGCTTCATCCGA

Supplementary Table 3. Antibodies used for immunofluorescence staining and western blot.

Antibody	Company	Code
ALDH3A1	Abcam	Ab76976
CD34	Santa Cruz	sc-9095
Lumican	Santa Cruz	sc-166871
Lumican	Abcam	ab168348
Keratocan	Santa Cruz	sc-66941
Collagen type I	Abcam	ab34710
Collagen type V	Santa Cruz	sc-166155
Alpha smooth muscle actin	Abcam	ab5694
β-Actin	Cell Signal	4967
Polyclonal Swine Anti-Rabbit Immunoglobulins/TRITC	Dako	R0156
Polyclonal Rabbit Anti-Mouse Immunoglobulins/TRITC	Dako	R0270
Anti-mouse IgG, HRP-linked Antibody	Cell Signal	7076
Anti-rabbit IgG, HRP-linked Antibody	Cell Signal	7074