cell cultures

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Abstract

Teeth were first disinfected with 75% ethanol and then washed with phosphate-buffered saline. Briefly, PDLSCs were separated from periodontal ligament in the middle one-third of the root. Subsequently, MSCs were digested in a solution of 3 mg/mL collagenase type I (Worthington Biochemical Corp., Lakewood, NJ, USA) and 4 mg/mL dispase (Roche Diagnostics Corp., Indianapolis, IN, USA) for 1 h at 37°C. Single-cell suspensions were obtained by cell passage through a 70-µm strainer (Falcon, BD Labware, Franklin Lakes, NJ, USA). MSCs were grown in a humidified, 5% CO_2 incubator at 37°C in DMEM alpha modified Eagle's medium (Invitrogen, Carlsbad, CA, USA), supplemented with 15% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 2 mmol/L glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). The culture medium was changed every 3 days. MSCs at passages 3-5 were used in subsequent experiments. Human embryonic kidney 293T cells were maintained in complete DMEM with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin.

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Protocol

Teeth preparation

Step 1.

Teeth were first disinfected with 75% ethanol and then washed with phosphate-buffered saline.

Tissue preparation

Step 2.

PDLSCs were separated from periodontal ligament in the middle one-third of the root.

cell digestion

Step 3.

MSCs were digested in a solution of 3 mg/mL collagenase type I (Worthington Biochemical Corp., Lakewood, NJ, USA) and 4 mg/mL dispase (Roche Diagnostics Corp., Indianapolis, IN, USA) for 1 h at 37°C.

Single-cell suspension

Step 4.

Single-cell suspensions were obtained by cell passage through a 70-µm strainer (Falcon, BD Labware,

Franklin Lakes, NJ, USA).

cell culture

Step 5.

MSCs were grown in a humidified, 5% CO_2 incubator at 37°C in DMEM alpha modified Eagle's medium (Invitrogen, Carlsbad, CA, USA), supplemented with 15% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 2 mmol/L glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen, Carlsbad, CA, USA). The culture medium was changed every 3 days. MSCs at passages 3-5 were used in subsequent experiments.