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Biological

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Mesenchymal Stem/Progenitor Cell Isolation from Tooth Extraction Sockets

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APPENDIX

MATERIALS & METHODS

Bone Fracture Model

After mice were anesthetized, an incision was created with scissors in the skin near the knee joint of the femur. A surgical incision with a blade was subsequently created from the medial portion of the knee joint to half the length of the femur. The femur was then exposed, separated from the muscles, and elevated with tweezers. Then, a fracture was induced *via* manual force application with the back portion of the blade holder. Finally, the surrounding muscle and joint were sutured followed by the overlying skin.

Isolation of Single Cells

Collected dog dental socket–derived cells and dog bone marrow–derived mesenchymal stem/progenitor cells were plated onto 10-cm culture dishes. After 2 wk, single-cell colonies were isolated with a plastic ring (6 mm in diameter × 9 mm in height) and detached with Accutase. The isolated cells were then plated into new plates, passaged to increase the cell count, and subsequently utilized for adipogenic and osteogenic differentiation assays.

Appendix Table 1. Primers Used for Real-time Reverse Transcription Polymerase Chain Reaction Analysis

Gene	GeneBank Accession No.	Primer Sequence	Product Length (bp)
s29[a]	NM_009093	5′-TTCCTTTCTCCTCGTTGG-3′ (S) 5′-ATGTTCAGCCCGTATTTG-3′ (AS)	108
Oct-4/Pou5f1[a]	AK145321	5′-GCATTGAGAACCGTGTGA-3′ (S) 5′-GATTGGCGATGTGAGTGAT-3′ (AS)	90
Nanog[a]	AK010332	5′-TTGGTGTGTTAGTGTATTTGTCT-3′ (S) 5′-TGGGAAGGAGAGAGTATATGC-3′ (AS)	101
GAPDH[b]	AB038240	5′-GCTGAGTATGTTGTGGAGTC-3′ (S) 5′-AGAAGGAGCAGAGATGATGA-3′ (AS)	102
ALP[b]	AF540075	5′-AGATGTGGAGTATGAGATGGA-3′ (S) 5′-CGTAGTGAGAGTGCTTGTG-3′ (AS)	110
LPL[b]	AY054979	5′-CTCTTTATTGACTCTCTGCTGAA-3′ (S) 5′-GGCTCTGACCTTATTGATCTC-3′ (AS)	147
COL2A1[b]	NM_001006951	5′-CAGCAAGAGCAAGGACAAG-3′ (S) 5′-AGTGGTAGGTGATATTCTGAGAG-3′ (AS)	164

S, sense; AS, antisense.
[a]Mouse.
[b]Dog.

Appendix Table 2. Percentage of Single Colonies of dDSCs and dBMSCs with Ability for Osteogenic and Adipogenic Differentiation, $N = 12$

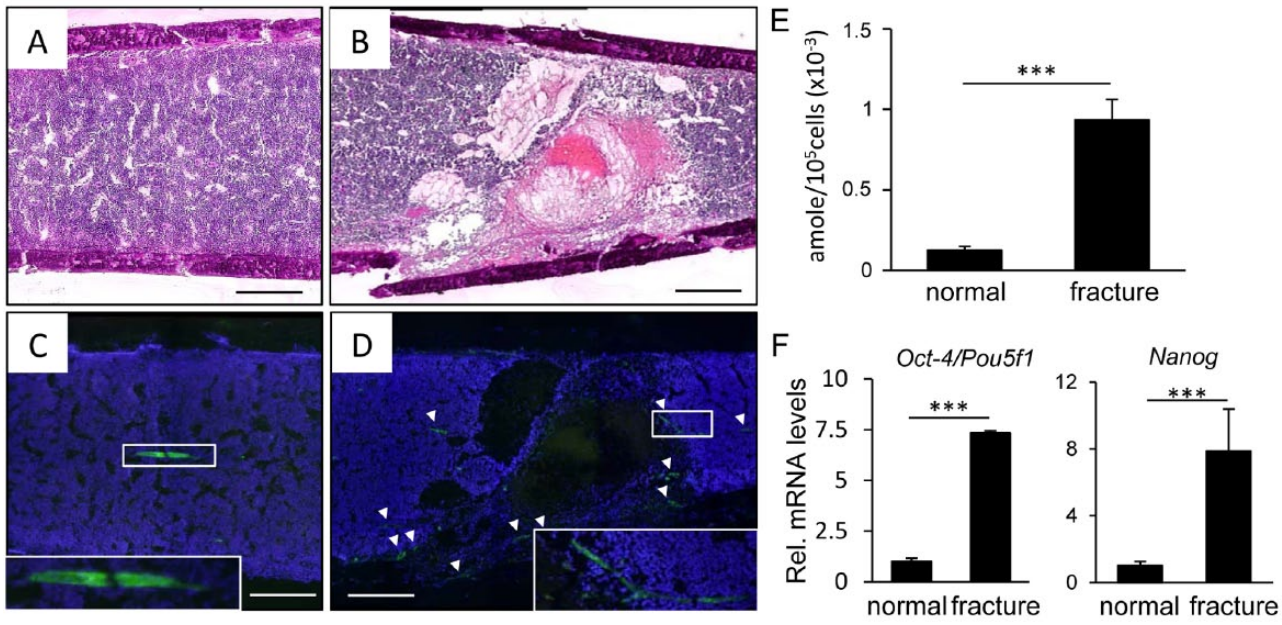
	Differentiation, % (*n*)		
	Osteogenic + Adipogenic	Osteogenic	Adipogenic
dDSCs	50 (6)	75 (9)	50 (6)
dBMSCs	50 (6)	50 (6)	50 (6)

dDSCs, dog dental socket–derived cells; dBMSCs, dog bone marrow–derived mesenchymal stem/progenitor cells.

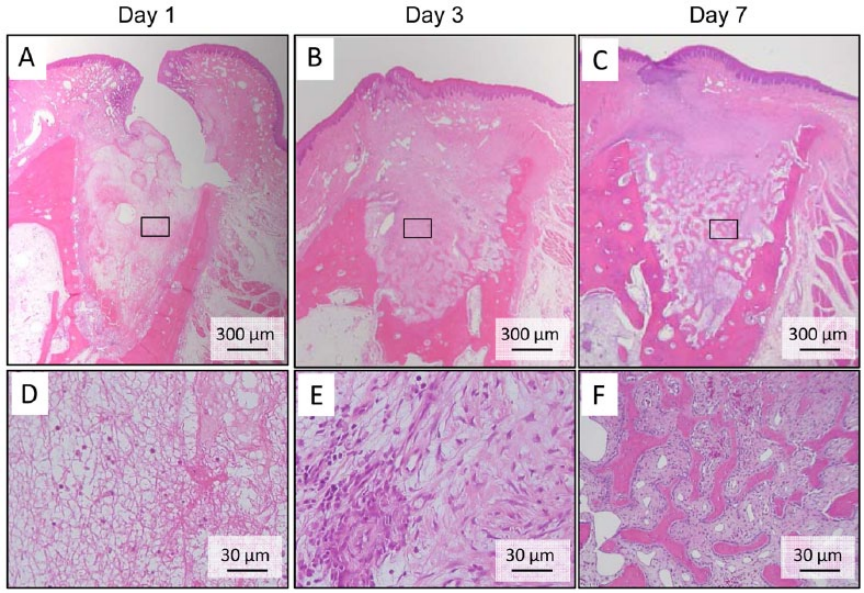
Appendix Table 3. Quantitative Analysis of New Bone and Periodontal Ligament Formation around the Tooth in a One-wall Defect Model in Dogs

	dDSCs (–)	dDSCs (+)
Ratio of regenerated bone, %	13.6 ± 7.8	25.8 ± 12.2
Length of regenerated periodontal ligament, cm	1.58 ± 0.76	2.94 ± 0.30*

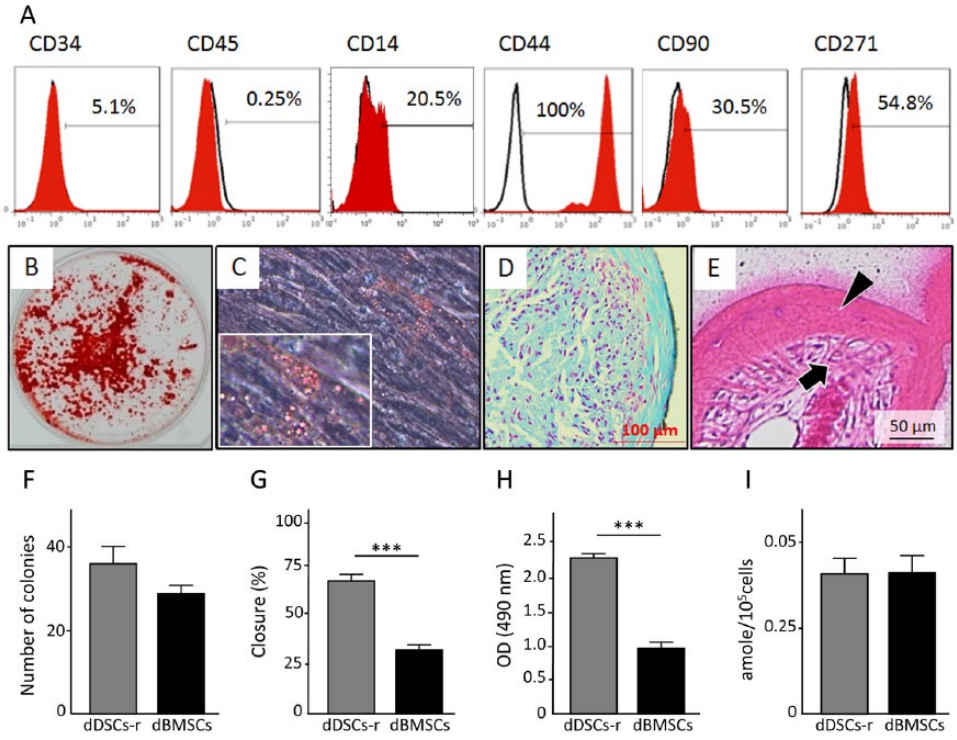
dDSCs, dog dental socket–derived cells.
*$p < .05$, compared with dDSCs (–). Student's *t* test.



Appendix Figure 1. Histologic analysis of the tissue and *in vitro* analysis of mouse bone marrow–derived mesenchymal stem/progenitor cells (mBMSCs) derived from the normal and fractured mouse femur. (**A, B**) Hematoxylin and eosin staining of normal (A) or fractured (B) femur. (**C, D**) Immunofluorescence staining for CD146 of normal mouse femur (C) or at 3 d after injury (D). (**E**) Telomerase activity of normal femur- and fractured femur–derived mBMSCs. (**F**) Comparative analysis of expression levels of *Oct-4* and *Nanog* in mBMSCs from normal and fractured femur.



Appendix Figure 2. Histologic analysis of the dog dental socket after tooth extraction. (**A-F**) Hematoxylin and eosin staining of dog dental sockets at 3 (A), 10 (B), and 14 d (C) after tooth extraction; the squares in Appendix Figure 2A, 2B, and 2C indicate the areas shown at higher magnification in Appendix Figure 2D, 2E, and 2F, respectively. Note that the dental socket was filled with fibrous tissue at 3 d, granulation tissue at 10 days, and trabecular bone at 14 d.



Appendix Figure 3. Characterization of dental socket–derived stem/progenitor cell–repeat (dDSC-r). (**A**) Surface molecule characterization of dDSCs-r. (**B-D**) Analysis of the multidifferentiation potential of dDSCs-r *in vitro* toward osteogenic (B; alizarin red S staining), adipogenic (C; oil red O staining), and chondrogenic (D; alcian blue staining) lineages. (**E**) Hematoxylin and eosin staining of the transplanted dDSCs-r/β-TCP sample. Similar to dental socket–derived stem/progenitor cells, dDSCs-r formed Sharpey's fiber-like tissue (arrow) connected with newly formed bone (arrowhead). (**F-I**) Analysis of the colony-forming ability (F), cell motility (G), proliferation (H), and telomerase activity (I). dDSCs-r showed a significantly higher degree of cell motility and proliferation than dog bone marrow stem/progenitor cells. All images and graphs are representative of 2 independent experiments.