COL4A2 in the tissue-specific extracellular matrix plays important role on osteogenic differentiation of periodontal ligament stem cells

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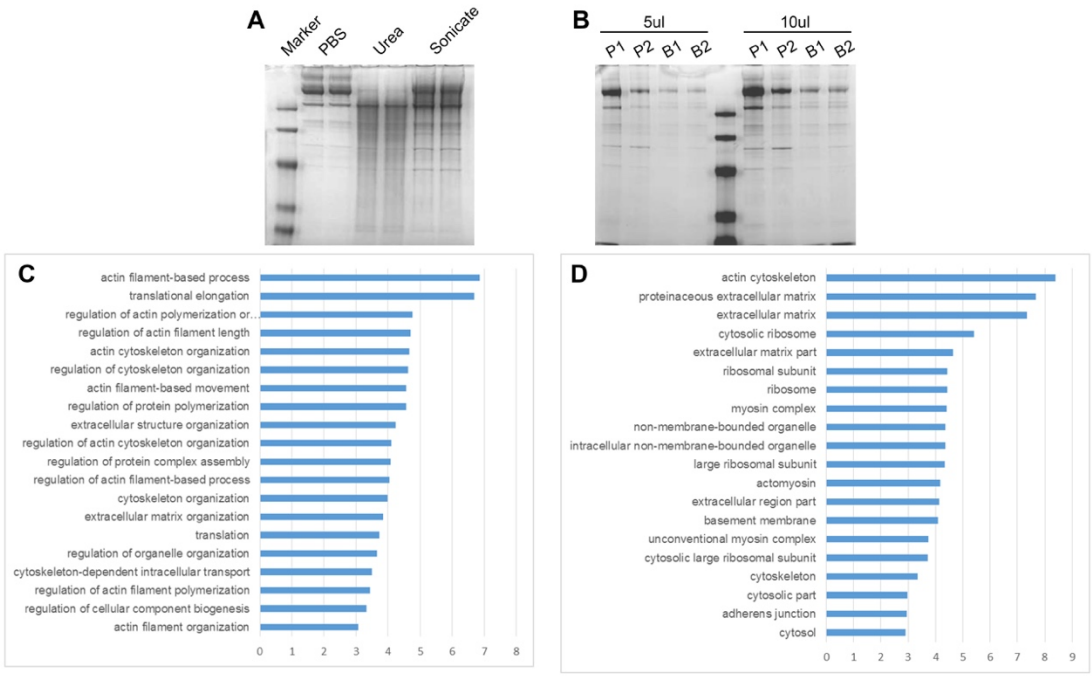
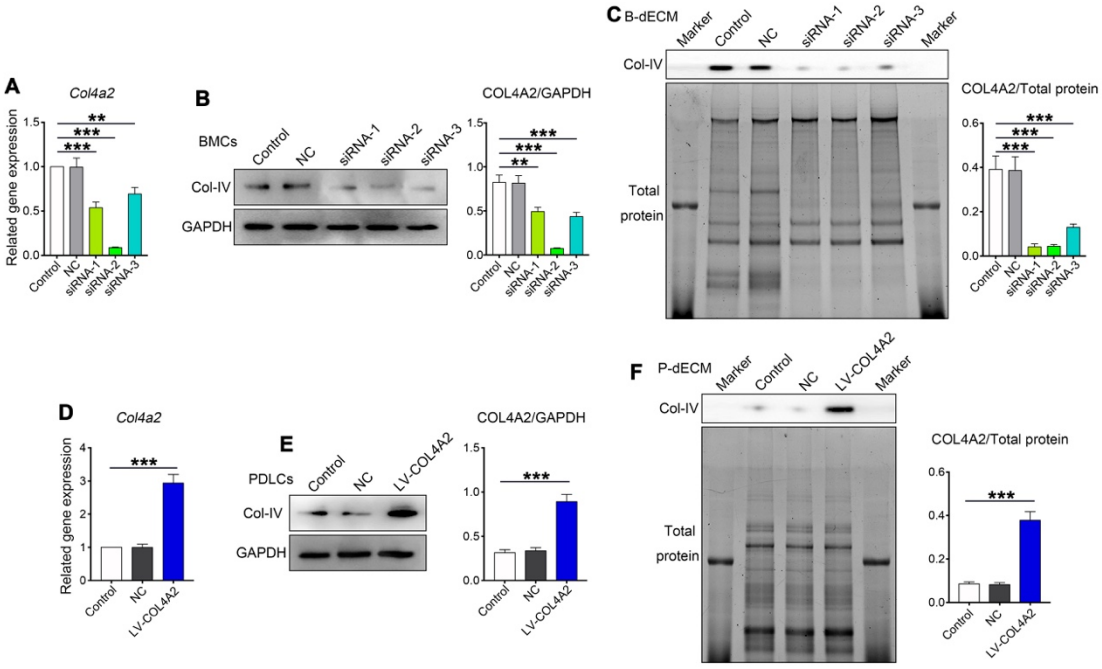
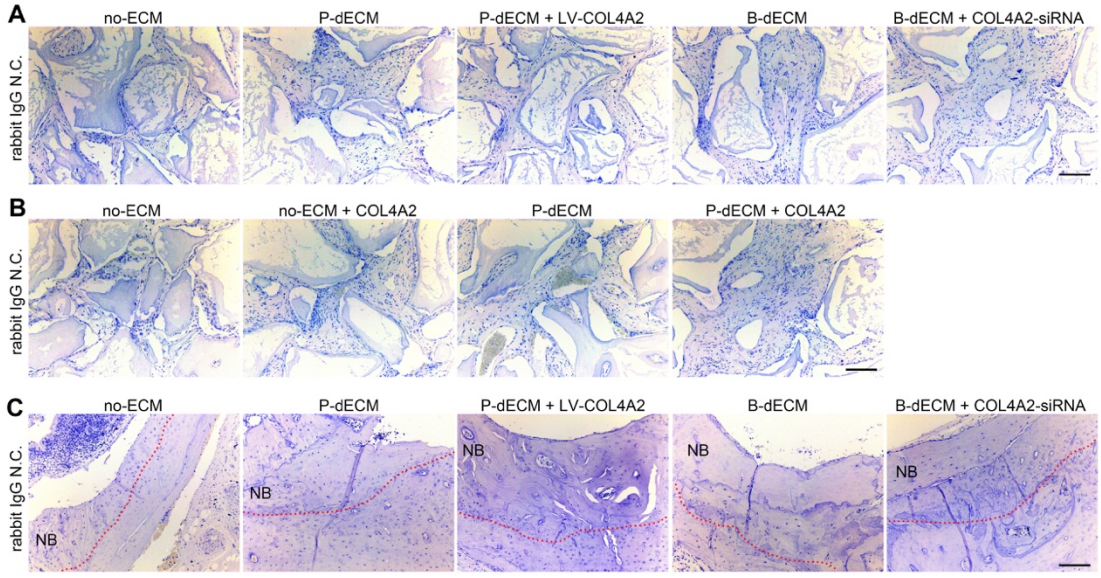
Supplementary Figures



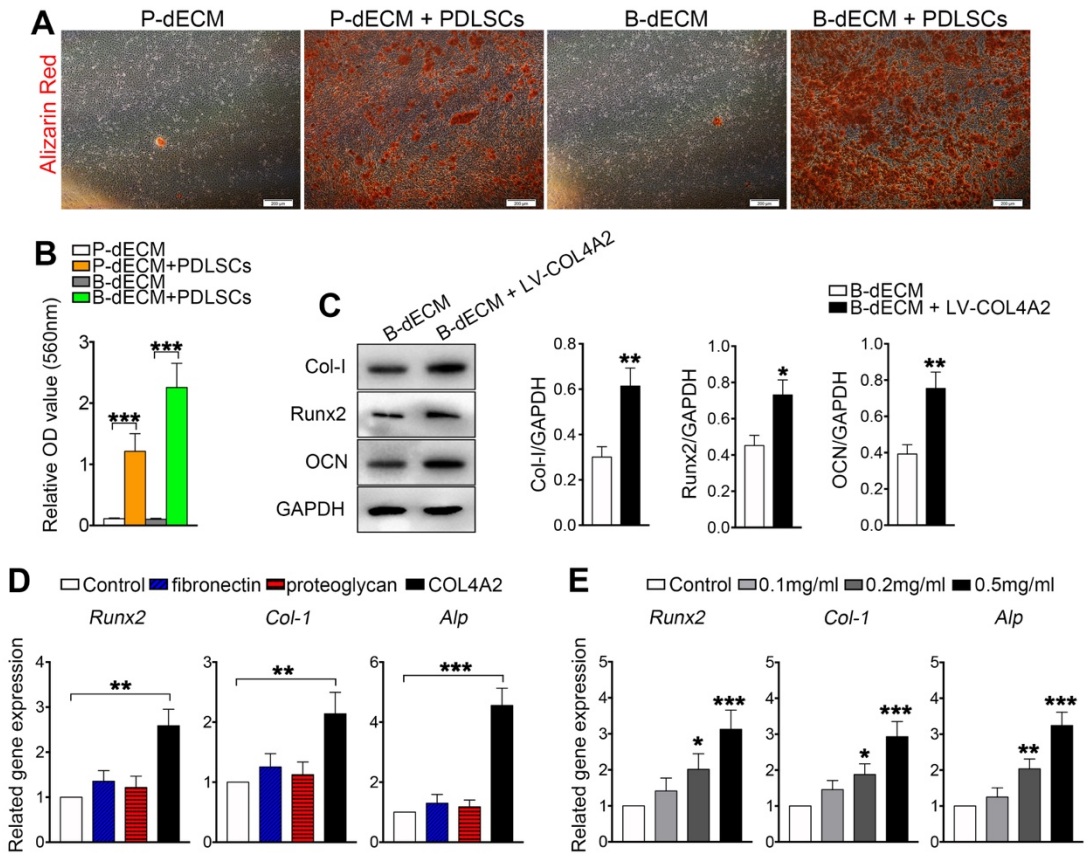
Supplementary Figure 1. LC-MS/MS analysis for protein mass spectrometry between P-dECM and B-dECM. (**A, B**) The quantification of the isolated proteins from different dECMs by western blot. After coomassie blue staining, the whole lanes of interest were excised and cut into small pieces. (**C**) Biological process of gene ontology in differential proteins between B-ECM and P-ECM. (**D**) Cellular component of gene ontology in differential proteins between B-ECM and P-ECM.



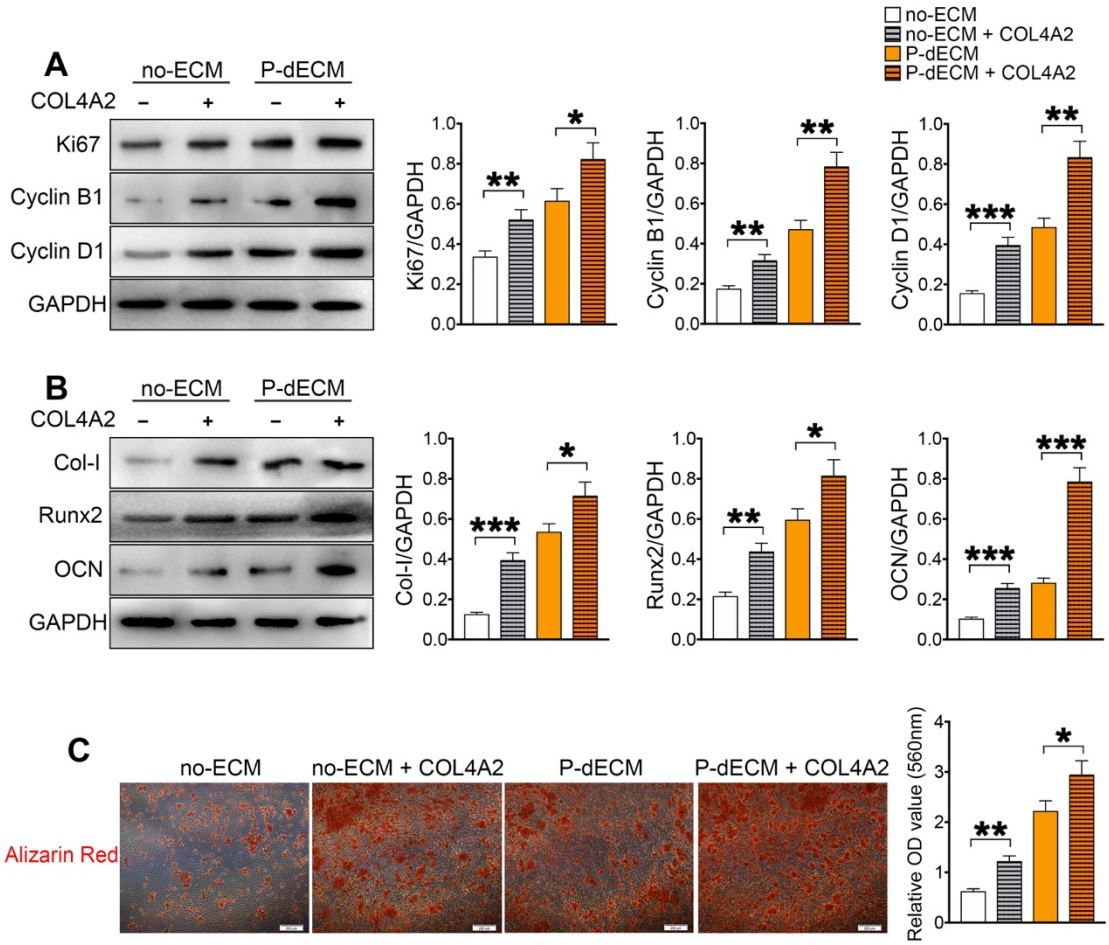
Supplementary Figure 2. The identification of the regulation of COL4A2 in dECM. (**A**) After the transfection of BMCs with siRNA to downregulate the COL4A2 expression, the mRNA expression of *Col4a2* was detected by qPCR. (**B**) The expression of COL4A2 in BMCs after downregulation compared with the control. The related quantification of blots (right panel). (**C**) The expression of COL4A2 in the decellularized ECM from BMCs (B-dECM) after the downregulation by siRNA. The total protein used as the internal inference. The related quantification of blots (right panel). (**D**) Through the lentivirus transfection to overexpression of *Col4a2* in PDLCs assayed by qPCR. (**E**) The expression of COL4A2 in PDLCs after upregulation compared with the control. The related quantification of blots (right panel). (**F**) The expression of COL4A2 in the decellularized ECM from PDLCs (P-dECM) after the upregulation by lentivirus. The total protein used as the internal inference. The related quantification of blots (right panel). *NC*, negative control. n = 5. ** $P < 0.01$ and *** $P < 0.001$ represent significant differences between the indicated columns.



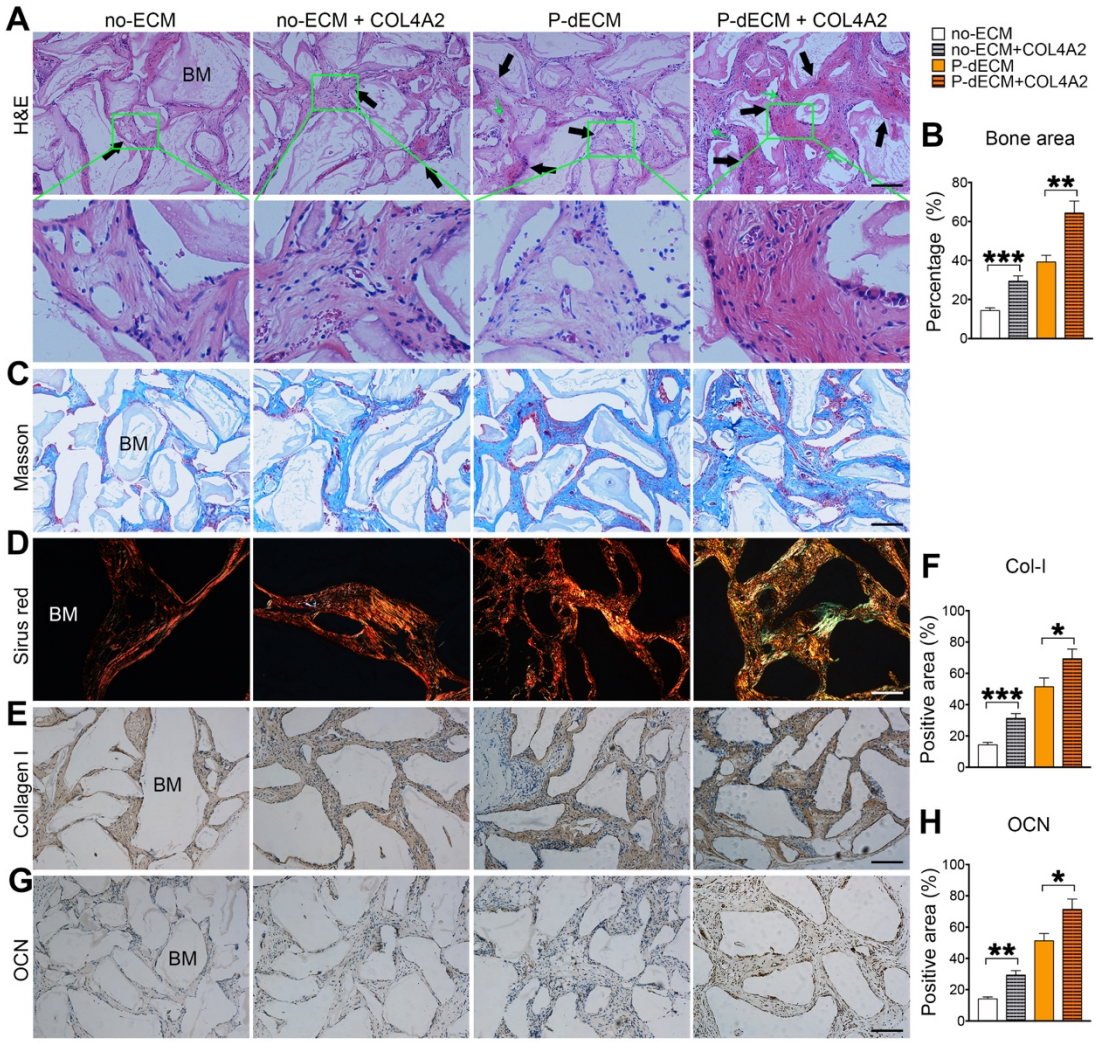
Supplementary Figure 3. The negative control of the IHC staining with rabbit antibodies. The sections were stained with IgG antibody and the anti-rabbit secondary and tertiary antibodies. Black bar, 200 μm.



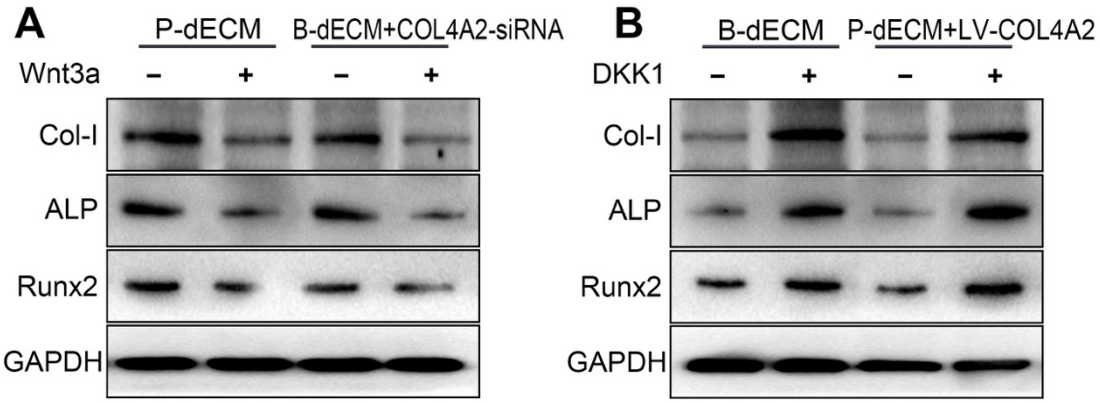
Supplementary Figure 4. Different ECM and material exists different osteogenic ability to PDLSCs. (**A**) Alizarin red staining for detecting the osteogenic differentiation ability of only dECMs and PDLSCs planted. (**B**) The quantification of (A). (**C**) Overexpression of COL4A2 in B-dECM influence the osteogenic differentiation ability of PDLSCs cultured in B-dECM the compared with B-dECM + LV-COL4A2. Western blot was used to detect the indicated protein expression for osteogenic differentiation of PDLSCs. The quantification of blots (right panel). (**D**) The culture dish coated with different material have influence to the PDLSCs culture for osteogenic differentiation ability in the osteogenic medium. The mRNA expression of *Runx2, Col-1* and *Alp* of PDLSCs in groups. (**E**) The mRNA expression of *Runx2, Col-1* and *Alp* of PDLSCs in different concentration of COL4A2 coated culture dish in the osteogenic medium. Data are presented as the means ± SD, n = 5. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ represent significant differences between the indicated columns.



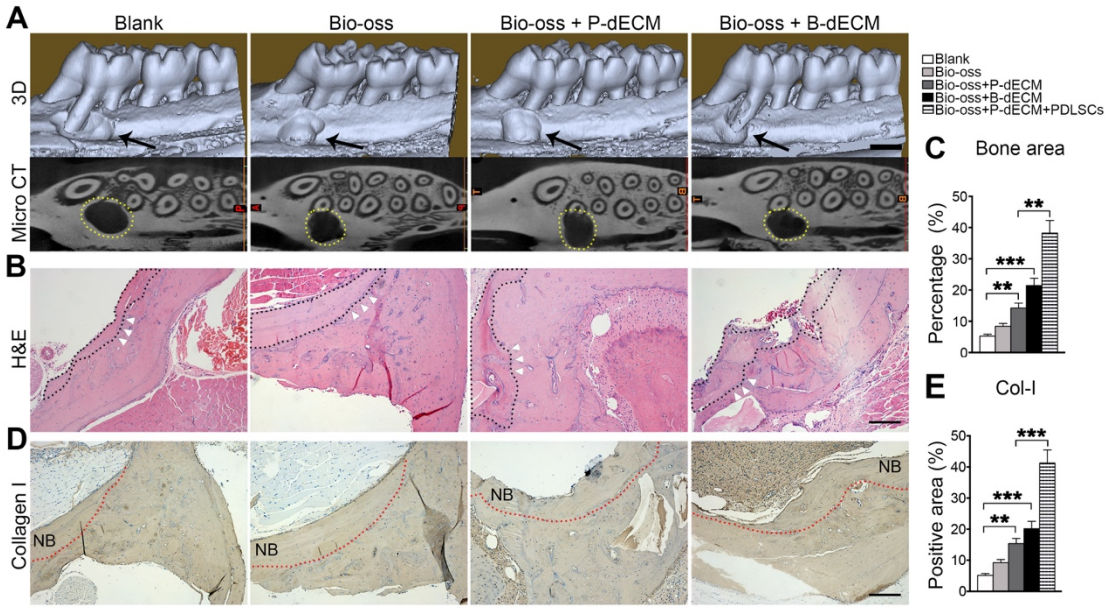
Supplementary Figure 5. Proliferation and osteogenic differentiation ability of PDLSCs cultured in the COL4A2 coated ECM. The TCP (no-ECM) and P-dECM were coated with COL4A2 peptide (0.5mg/ml). (**A**) The protein expression of Ki67, Cyclin B1 and Cyclin D1 of PDLSCs cultured on specific coated dECMs compared with no-ECM in the culture medium. The quantification of blots (right panel). (**B**) Western blot was used to detect the indicated protein expression for osteogenic differentiation of PDLSCs cultured on specific coated dECMs in the osteogenic medium. The quantification of blots (right panel). (**C**) Alizarin red staining for detecting the osteogenic differentiation ability of PDLSCs in four indicated groups in the osteogenic medium. The quantification of positive staining (right panel). Data are presented as the means ± SD, n = 5. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ represent significant differences between the indicated columns.



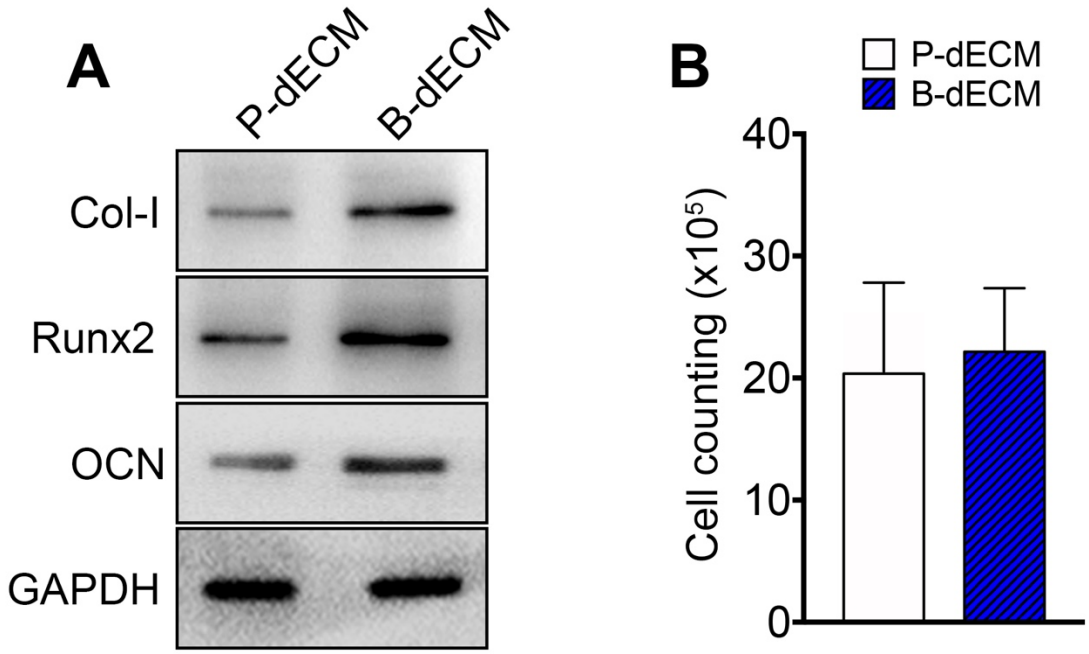
Supplementary Figure 6. New bone-like tissue around the Bio-Oss bone powder in immunocompromised mice through PDLSCs implantation with COL4A2 coated ECM. The P-dECM or TCP (no-ECM) were coated with COL4A2 peptide (0.5mg/ml). (**A**) H&E staining revealed that more bone-like tissue and inserting PDL-like fibers were observed in no-ECM + COL4A2 or P-dECM + COL4A2 than no-ECM or P-dECM groups respectively. *Black arrow*, the newly formed bone-like tissue. *Green arrow*, the newly formed vessels. (**B**) Quantitative analysis of the new bone area on H&E staining images. (**C**) Masson staining for the new bone and fibers. (**D**) Sirius red staining for the identification of fibers from collagen I to IV. Collagen I, red birefringence; collagen II, weak red light; collagen III, green birefringence; collagen IV, weak yellow birefringence. (**E, F**) Immunohistochemical staining of Col-I and the quantification of the positive area of Col-I. (**G, H**) Immunohistochemical staining of OCN and the quantification of the positive area of OCN. *BM*, bone meal from Bio-Oss. Black bar and white bar, 200 μm. Data are presented as the means ± SD, n = 6. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ represent significant differences between the indicated columns.



Supplementary Figure 7. Osteogenic ability through COL4A2 in ECMs is negative regulated by the modulation of Wnt pathway. (**A**) Activation of Wnt/β-catenin by Wnt3a in the P-dECM and B-dECM + COL4A2-siRNA group. Western blot was used to detect the indicated protein expression for osteogenic differentiation of PDLSCs. (**B**) Inhibition of Wnt/β-catenin by DKK1 in the B-dECM and P-dECM + LV-COL4A2 group. Western blot was used to detect the indicated protein expression for osteogenic differentiation of PDLSCs.



Supplementary Figure 8. Characterization of different specific dECMs repair effect of alveolar bone defects in rats at 8-weeks. The blank control has none of any transplantation. Bio-Oss group means the only transplantation of Bio-Oss material. P-dECM or B-dECM mixed with Bio-Oss implanted into the defects for the related detection. (**A**) Representative images of Micro-CT 3D images and horizontal cross section for the above four indicated groups. *Black arrow*, the size of the alveolar bone defects after transplantation. *Yellow dots*, the size of the alveolar bone defects after implantation in the horizontal cross section. Black bar, 1 mm. (**B**) H&E staining of bone defects in these four groups. *Black dots*, the area of the new bone in defects. *Green arrow*, newly formed blood vessels. Black bar, 200 μm. (**C**) Quantitative analysis of the new bone area on H&E analysis in these four groups compared with the PDLSCs implantation groups. (**D**) Immunohistochemical staining of Col-I of alveolar bone defects treated with dECMs or Bio-Oss compared with blank control after 8-weeks transplantation *NB*, new bone. *Red dots*, the area of the new bone in defects. Black bar, 200 μm. (**E**) The related quantification of (D) in these four groups compared with the PDLSCs implantation groups. Data are presented as the means ± SD, n = 6. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ represent significant differences on the indicated columns.



Supplementary Figure 9. Characterization of osteogenic differentiation ability of PDLSCs in different dECMs. 1.5×10^6 PDLSCs were seeded onto B-dECM and P-dECM and cultured in the osteogenic medium to avoid further proliferation. (**A**) Protein expression of Col-I, Runx2 and OCN assayed by Western blotting. (**B**) The cell counting in different groups after 7 days' culture.

Supplementary Table 1. Human gene primers.

Genes	Forward primer	Reverse primer
Cyclin D1	CCCCTTCCATCTCTGACTTA	CCTCTATCATCTGTAGCACAACC
Sox-2	CATCACCCACAGCAAATGACA	GCTCCTACCGTACCACTAGAACTT
Nanog	CCTGTGATTTGTGGGCCTGA	CTCTGCAGAAGTGGGTTGTTTG
Oct-4	GCAGCGACTATGCACAACGA	CCAGAGTGGTGACGGAGACA
Runx2	CACTGGCGCTGCAACAAGA	CATTCCGGAGCTCAGCAGAATAA
Alp	GGACCATTCCCACGTCTTCAC	AACCAAGCTTTGTGCCTTCACTTC
Ki67	AATGCACACTCCACCTGTCCTG	AACCAAGCTTTGTGCCTTCACTTC
Ocn	CCCAGGCGCTACCTGTATCAA	GGTCAGCCAACTCGTCACAGTC
Pparγ	CTCCTATTGACCCAGAAAGC	GTAGAGCTGAGTCTTCTCAG
Lpl	GGGCATGTTGACATTTACCC	AGCCCTTTCTCAAAGGCTTC
Cebp	CCCGGCAACTCTAGTATTTAGG	AATGACAAGGCACGATTTGC
Fabp	TCATACTGGGCCAGGAATTTGACA	ATGCGAACTTCAGTCCAGGTCAAG
Col1	CCAGAAGAACTGGTACATCAGCAA	CGCCATACTCGAACTGGAATC
Col4a2	CGGAGTTTGTGGATCGGATA	GCATTCGATGAATGGTGTGG
Col2a1	CCAGTTGGGAGTAATGCAAGGA	ACACCAGGTTCACCAGGTTCA
Acan	ACGAAGACGGCTTCCACCAG	TCGGATGCCATACGTCCTCA
Gapdh	AGCCGCATCTTCTTTTGCGTC	TCATATTTGGCAGGTTTTTCT