doi: 10.1387/ijdb.120076hl

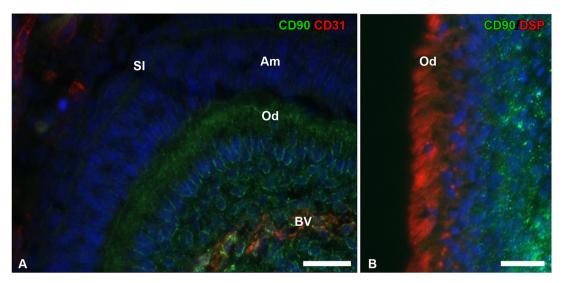


SUPPLEMENTARY MATERIAL

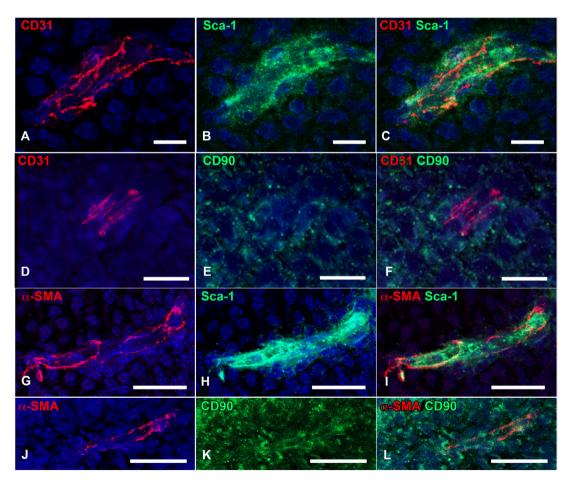
corresponding to:

Restoring physiological cell heterogeneity in the mesenchyme during tooth engineering

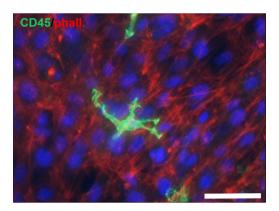
LAETITIA-VÉRONIQUE KELLER, SABINE KUCHLER-BOPP and HERVÉ LESOT



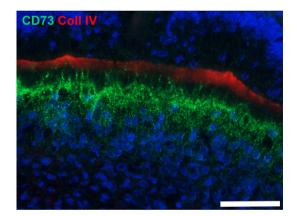
Supplementary Fig. S1. Transient expression of CD90 by odontoblasts during molar development. CD90, in green, was expressed by odontoblasts at PN1 (A), but no longer detected at PN4 (B). Am: Ameloblast; BV: Blood vessel; Od: Odontoblast; SI: Stratum intermedium. Scale bars: 30 µm.



Supplementary Fig. S2. Confocal microscopy observations of cells in immediate contact with blood vessels of engineered tooth organ. Double immunostainings for CD90 (D-F,J-L) or Sca-1 (A-C,G-I) with either CD31 (A-F) or α -SMA (G-L) in re-associations implanted for 2 weeks. α -SMA positive cells can also be positive for Sca-1 (G-I) and CD90 (J-L). Similarly, some cells can be labeled for both CD31 and Sca-1 (A-C). Scale bars: 30 μ m.



Supplementary Fig. S3. Expression of CD45 in mesenchymal dental single cell cultured for 4 days as a monolayer. Cultured mesenchymal cells positive for CD45 exhibit a typical dendritic shape. CD45 positive cells are visualized by green fluorescence and others cells are visualized by staining for actin, using phalloidine. Scale bar: $50 \mu m$.



Supplementary Fig. S4. Expression of CD73 in early bell stage molar cultured for 7 days in semi-solid medium. Although CD73 was not expressed at the onset of the culture, this antigen was strongly expressed by odontoblasts of ED18 molars cultured for 7 days. Scale bar: 50 µm.

TABLE S5

EXPRESSION OF CELL SURFACE MARKERS IN THE DENTAL MESENCHYME OR DENTAL MESENCHYMAL CELLS IN THE DIFFERENT STEPS OF OUR PROTOCOL FOR TOOTH ORGAN ENGINEERING AND COMPARISON WITH STAININGS AT DIFFERENT STAGES OF TOOTH DEVELOPMENT

	CD31 Blood vessels	CD34 Blood vessels	CD146 Blood vessels + pericytes	Sca-1 Hematopoietic stem cell + Blood vessels	α–SMA Pericytes	CD45 Hematopoietic stem cells	CD90 Mesenchymal stem cells	CD73 Mesenchymal stem cells
ED14 molar in situ	+	+	+	-	-	-	+	-
Isolated ED14	+	+	+	-	-	-	-	-
Cultured mesenchymal single cells	-	+	+	+	ND	+	+	+
ED18 molar in situ	+	+	+	-	-	+	+	-
R14+8	-	-	-	-	-	+	+	-
PN1 molar in situ	+	+	+	+	-	+	+	-
PN4 molar in situ	+	+	+	+	+	+	+	+
R14+8+2w	+	+	+	+	+	+	+	+
R14+8+2w GFP+	+	+	+	+/-	+	-	+/-	-

The cell surface markers observed by immunofluorescence are commonly used to select or to observe blood vessels, pericytes, hematopietic stem cells or mesenchymal stem cells. The symbol (+) was used when the expression was found in the dental mesenchyme, while the symbol (-) was used when no expression was found in this compartment. After implantation of cell re-associations in GFP mice, (+) was used for cells co-expressing GFP and a cell surface marker, (-) was used when no co-expression could be visualized, and (+/-) represents cells expressing the cell surface marker, which could be either positive or negative for GFP. ND: Not Determined.