Appendix A

Table A. List of antibodies with their specific reactivity and the dilution used to characterize the cell composition of equine blood and to further characterize equine mesenchymal stromal cells. Cells declared as non-specific were excluded from our study.

Antibody	Reactivity	Dilution	Comments	Refs.
Rat anti horse CD3:FITC, clone CD3-12, 1 mg/ml, Abcam plc.	horse-specific	1:20	specific binding, intracellular binding	[1]
Mouse anti horse CD8:PE, clone CVS21, 1 mg/ml, Bio-Rad AbD Serotec GmbH	horse-specific	1:20	specific binding	[2]
Mouse anti horse CD4:FITC, clone CVS4, 1 mg/ml, Bio-Rad AbD Serotec GmbH	horse-specific	1:500	specific binding	[2]
Mouse anti horse CD14:APC, clone 105, unknown conc., Cornell University	horse-specific	1:2.000	specific binding	[3]
Mouse anti equine Granulocytes:VioBlue, clone HACT39A, 1mg/ml, Kingfisher Biotech Inc.	horse-specific	1:1.200	specific binding, conjugation in house	-
Mouse anti horse CD16:PerCP, clone KD1, 1 mg/ml, Abcam plc.	horse-specific	-	non-specific binding, conjugation in house	[4]
Mouse anti human CD21, clone Bu33, 1 mg/ml Bio-Rad AbD Serotec GmbH	cross-reactive	-	non-specific binding	[5]
Mouse anti horse pan B-Cells:RPE, clone CVS36, 1 mg/ml Bio-Rad AbD Serotec GmbH	horse-specific	-	non-specific binding	-
Mouse anti human CD105:Alexa Fluor® 488, clone SN6, 1 mg/ml Bio-Rad AbD Serotec GmbH	cross-reactive	1:40	specific binding	[6]
Mouse anti human Integrin beta1/CD29:PE, clone 419127, 25 µg/ml, Bio-Techne GmbH	cross-reactive	1:20	specific binding	[7]
Mouse anti horse CD44:RPE, clone CVS18, 1 mg/ml, Bio-Rad AbD Serotec GmbH	horse-specific	-	non-specific binding	[8]
Mouse anti horse CD90:DyLight 405®, clone NS1, 1 mg/ml, Abcam plc.	horse-specific	(-)	specific binding, conjugation in house	[9]

Table B. Depicted is the data of the equine donors both for the blood clots and the *in vitro* fracture hematoma models. For the blood clots the blood of all 4 donors was coagulated (n=4). For the creation of the *in vitro* FH models either blood 1, 2 or 3 was mixed according to the protocol as described above with the MSC of the donor MSC1 (n=3).

Sample Name/Number	Age (years)	Sex	Race
Blood 1	6	mare	Trotter
Blood 2	14	gelding	Trotter
Blood 3	26	mare	Arabian horse
Blood 4	22	gelding	Icelander
MSC 1	13	gelding	Riding horse

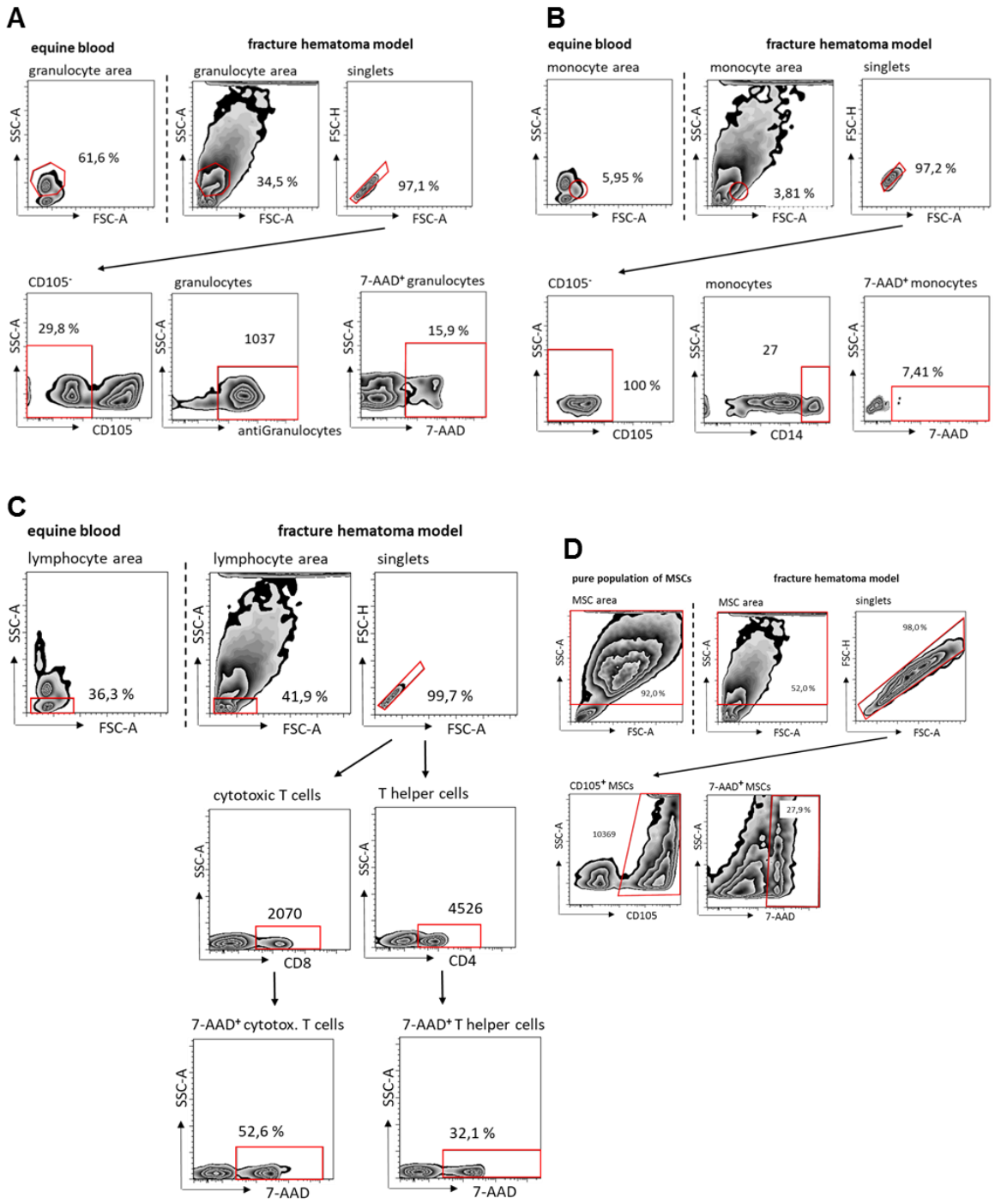

Fig A. Gating strategy for the flow cytometric characterization of cells (MSCs, granulocytes, monocytes, CD4+, and CD8+ cells), within the blood coagulates as well as in the *in vitro* FH models. (A) Gating strategy for the granulocyte population concerning their granularity and size, as well as the anti-granulocyte marker and the absence of the surface marker CD105 (to exclude MSCs). (B) Gating strategy of the monocyte population regarding the expression of CD14 and the lack of expression of CD105. (C) Gating of the lymphocyte fraction and concerning their typical surface markers T-helper-cells (CD4+) and cytotoxic T-cells (CD8+). (D) Gating of the MSC population regarding the typical surface marker CD105.

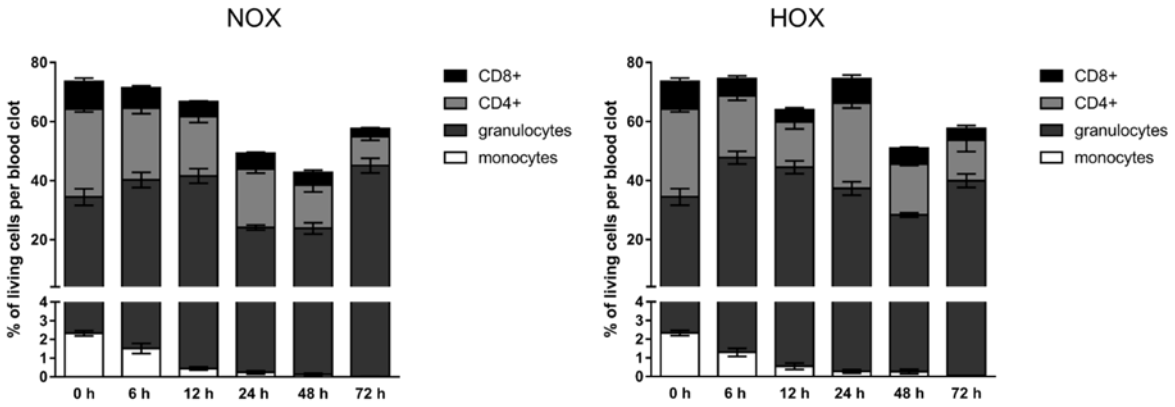

Fig B. Frequency of immune cell populations in blood clots without MSC. Frequency of granulocytes, CD14+ monocytes, CD4+ T cells, CD8+ T cells negative for 7-AAD present in the blood clots as incubated in osteogenic differentiation medium under either hypoxic or normoxic conditions (37 °C, 5% CO2, 18%/ 1% O_2) for 6, 12, 24, 48, and 72 h (mean ± SEM, n = 4).

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