

Title: An insight into the stages of ion leakage during red blood cell storage

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Cell Name	Diameter 1 - Diameter 2 [μm]	Height 1 - Height 2 [μm]	Height / Diameter	Spicules
Discocyte	≤ 0.2	≤ 0.3		no
Eliptocyte	≥ 1.0			no
Codocyte			> 0.35	no
Stomatocyte	< 1.0			no
Echinocyte				yes
Spherocochinocyte			> 0.35	yes
Spherocyte			> 0.35	no

Table S1. Table with cell naming criteria used for analysis of RBCs morphological changes during 6 week storage.

Ions	Extracellular [mmol/L]	Intracellular [mmol/L]
[Na ⁺]	135-145	10
[Cl ⁻]	95-105	3
[K ⁺]	3.8-5.5	159
[HCO ₃ ⁻]	22-28	7
[H ⁺]	$36 \times 10^{-6} - 44 \times 10^{-6}$	63×10^{-6}

Table S2. Reference ranges of extracellular and intracellular concentrations of Na⁺, Cl⁻, K⁺, HCO₃⁻ and H⁺ ions in human blood in adults. Zapala B. et al. Laboratory Diagnostics with Elements of Clinical Biochemistry. Book, 2017:874-886; Casey JR, et al. Nat Rev Mol Cell Biol. 2010;11(1):50-61; Rhoades R. et al. Medical Physiology : Principles for Clinical Medicine. Book 2009.

Parameter	Unit	Reference range
Lactates	mmol/L	0.6-2.2
Glucose*	mmol/L	3.9-5.5
free Fe	μmol/L	♂ 14-32
		♀ 11-29
RBC	mln/μL	♂ 3.91-5.11
		♀ 3.91-5.61
HGB	g/dL	♂ 13.2-17.3
		♀ 11.7-15.5
HCT	%	♂ 38-51
		♀ 33-45
MCV	fL	86-98
MCH	pg	28-33
MCHC	g/dL	32-36

*fasting

Table S3. Reference ranges of main biochemical (lactates, fasting glucose, free Fe) and morphological (RBC, HGB, HCT, MCV, MCH, MCHC) parameters in adults, with a division based on gender (♂ - males, ♀ - females) for free Fe, RBC, HGB and HCT values. Zapala B. et al. Laboratory Diagnostics with Elements of Clinical Biochemistry. Book, 2017:874-886.

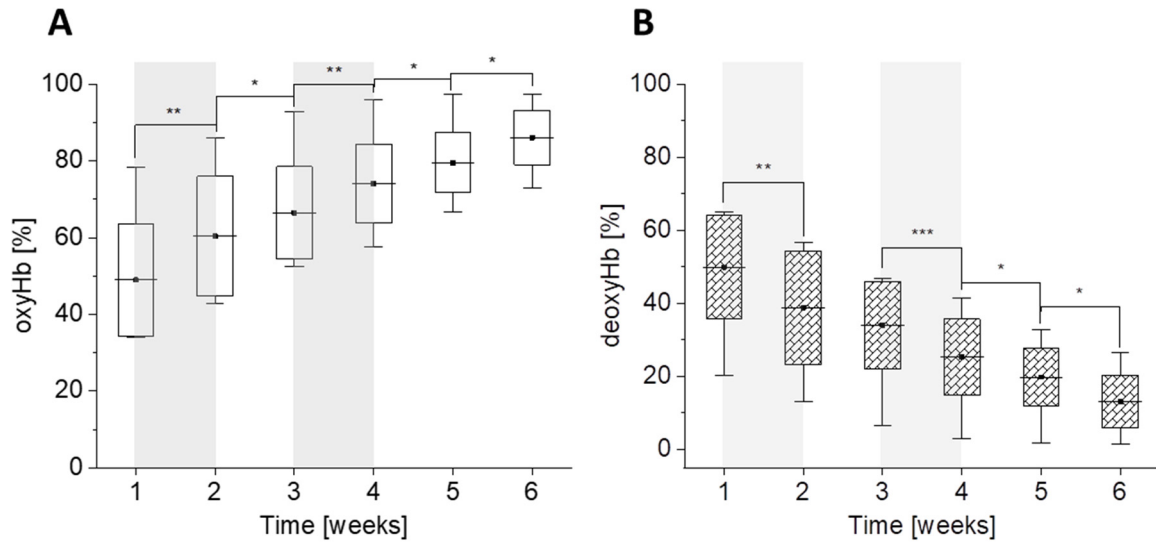


Figure S1. Percentage changes of the oxyHb (**A**) and deoxyHb (**B**) during storage pRBCs. The most prominent changes are marked gray. Data distribution is presented as box plots (mean value, mean \pm SD, min-max). Statistical significance of the obtained values was tested with Kruskal-Wallis ANOVA nonparametric test (null = not significant;; * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

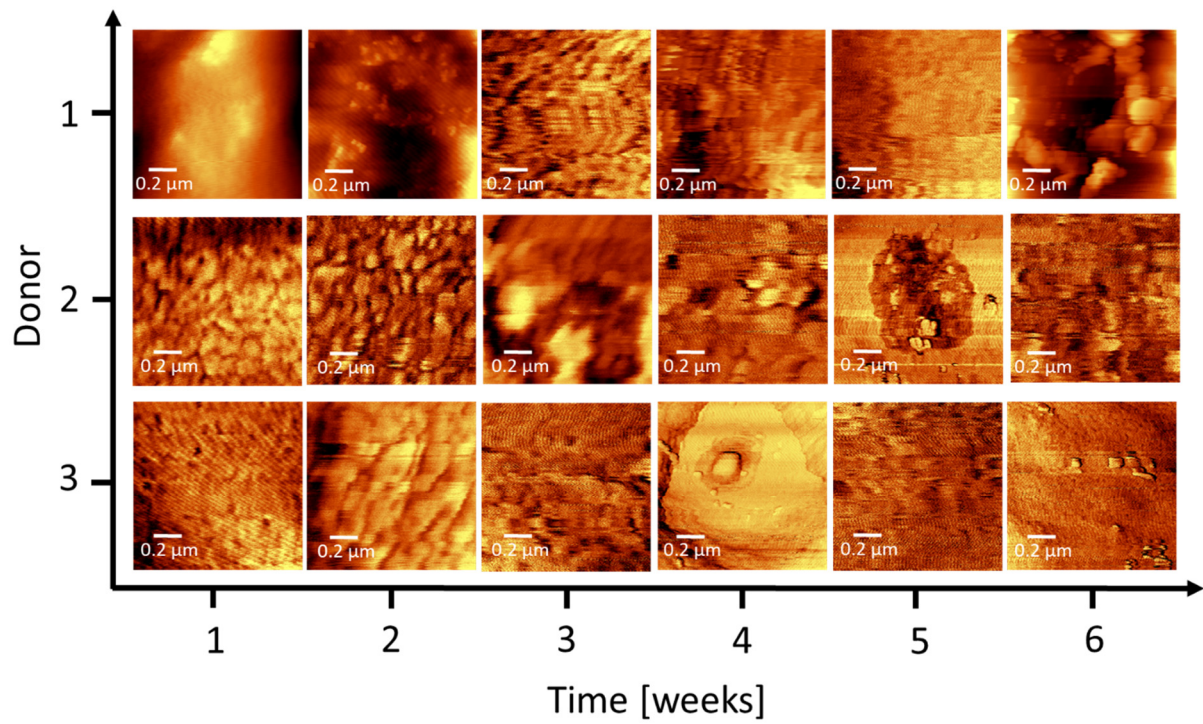


Figure S2. Representative nanoscale AFM images of RBCs' surface changes collected in scale $1.5 \times 1.5 \mu\text{m}^2$ during 6 weeks storage of the pRBCs for three donors.