Online Supplementary Content

Donors' characteristics

Table SI - Donors' characteristics.

Sample	Blood type	Sex	Age of donor, years
EC 1	O+	Female	58
EC 2	O−	Female	43
EC 3	A+	Male	50
EC 4	O+	Male	49
EC 5	O+	Male	33

Composition of HEPA buffer

HEPA 10 mM glucose was prepared at the beginning of the follow up and stored at 4 °C. It was filtrated at 0.22 µm before use. It is composed of: NaCl 130 mM, KCl 5.4 mM, $CaCl_2{\cdot}2H_2O$ 1 mM, $MgCl_2{\cdot}6H_2O$ 0.5 mM, glucose 10 mM, Hepes 15 mM, BSA 1 mg/mL. pH was adjusted at 7.4 with NaOH or HCl, theoretical osmolarity is approximately 298 mOsm. Chemicals came from MSD Merck Sharp & Dohme, Luzern, Switzerland, and Sigma-Aldrich, Steinheim, Germany.

Details on cell membrane fluctuations measurements

Fluctuations rate can be measured using Equation (1).

$$\mathrm{Var}(\varphi_{cell} + \varphi_{background}) = \mathrm{Var}(\varphi_{cell}) + \mathrm{Var}(\varphi_{background}) + 2\mathrm{Cov}(\varphi_{cell} + \varphi_{background}) \quad (1)$$

The temporal deviation of each pixel at $(i, j)^{th}$ position can be measured using Equation (2):

$$\mathrm{std}(\varphi_{cell})_{(i,j)} = \sqrt{[\mathrm{std}(\varphi_{cell} + \varphi_{background})_{(i,j)}]^2 - [\mathrm{std}(\varphi_{background})]^2} \quad (2)$$

Eventually, the CMF is measured with Equation (3):

$$\mathrm{CMF}_{OPD}(nm)_{(i,j)} = \mathrm{std}(\varphi_{cell})_{(i,j)} \quad (3)$$

in which $\mathrm{Var}(\varphi_{cell})$ and $\mathrm{Var}(\varphi_{background})$ are the temporal variance in OPD corresponding to the CMF and to the background fluctuations, respectively, and $\mathrm{Cov}(\varphi_{cell}, \varphi_{background})$ is the covariance of the two variables. Assuming that the two variables are independent, $\mathrm{Cov}(\varphi_{cell}, \varphi_{background})=0$.

All the simulations were implemented in Matlab 2015.

Time course changes in intracellular oxidised glutathione

Intracellular GSSG concentration is presented in Figure S1.

Correlation between population and single-cell analyses with digital holographic microscope

The correlation between red blood cell (RBC) morphology (based on CPA) and the SD-OPD value demonstrated that SD-OPD was linearly correlated to the percentage of spherocytes (positively, R^2=0.98) and discocytes (negatively, R^2=0.98) in the sample (see Figure S2), but not to the percentage of stomatocytes and echinocytes.

Single-cell morphology analysis (CellProfiler and CPA) for each individual erythrocyte concentrate

Results of single-cell morphology analysis (CellProfiler and CPA) are presented for each individual EC in Figure S3.

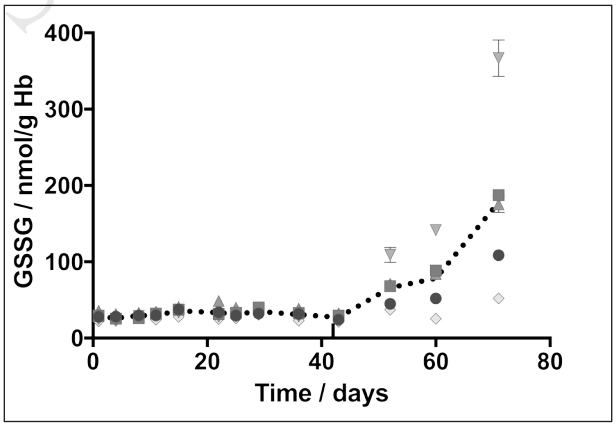

Figure S1 - Intracellular GSSG concentration for ECs 1-5 stored during 71 days.
Individual (symbols) and mean values (dotted line) are presented ± standard deviation. GSSG: oxidised glutathione.

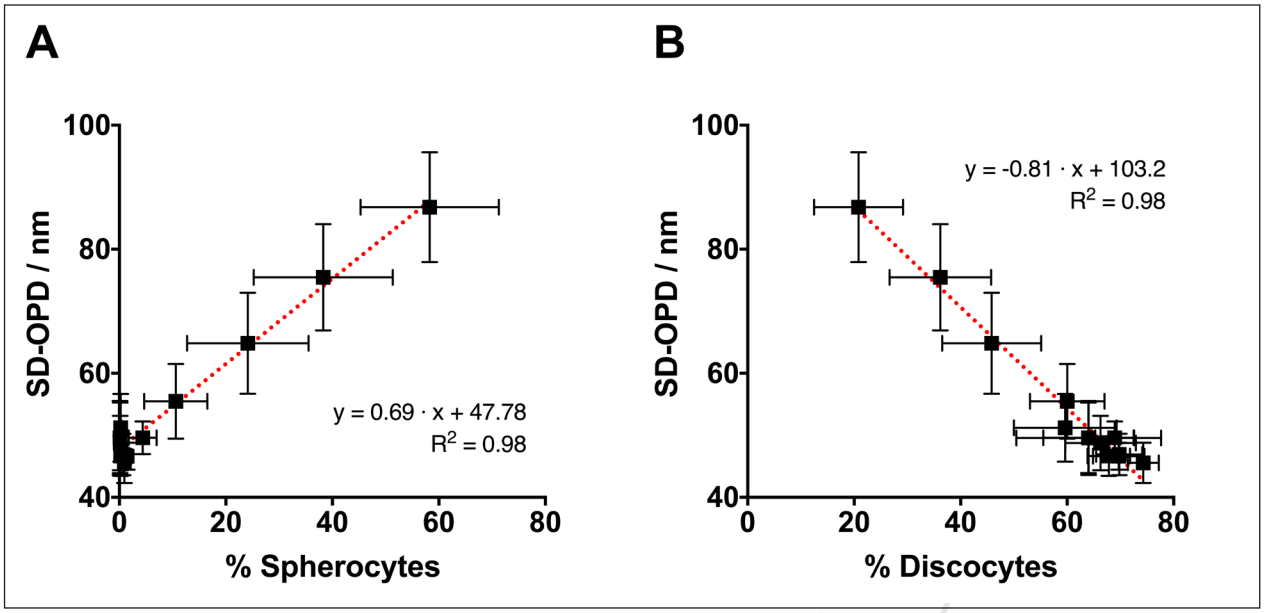

Figure S2 - Correlation between SD-OPD (nm) and percentage of spherocytes (A) and discocytes (B) in the sample obtained with CellProfiler analysis at each time point, mean values are presented ± standard deviation.
DHM images of RBCs were acquired weekly over 71 days at a 20× magnification. The equation of linear correlation curve (dotted line) and regression coefficient R^2 are specified. SD-OPD: standard deviation of the optical path difference distribution; DHM: digital holographic microscope; RBC: red blood cell.

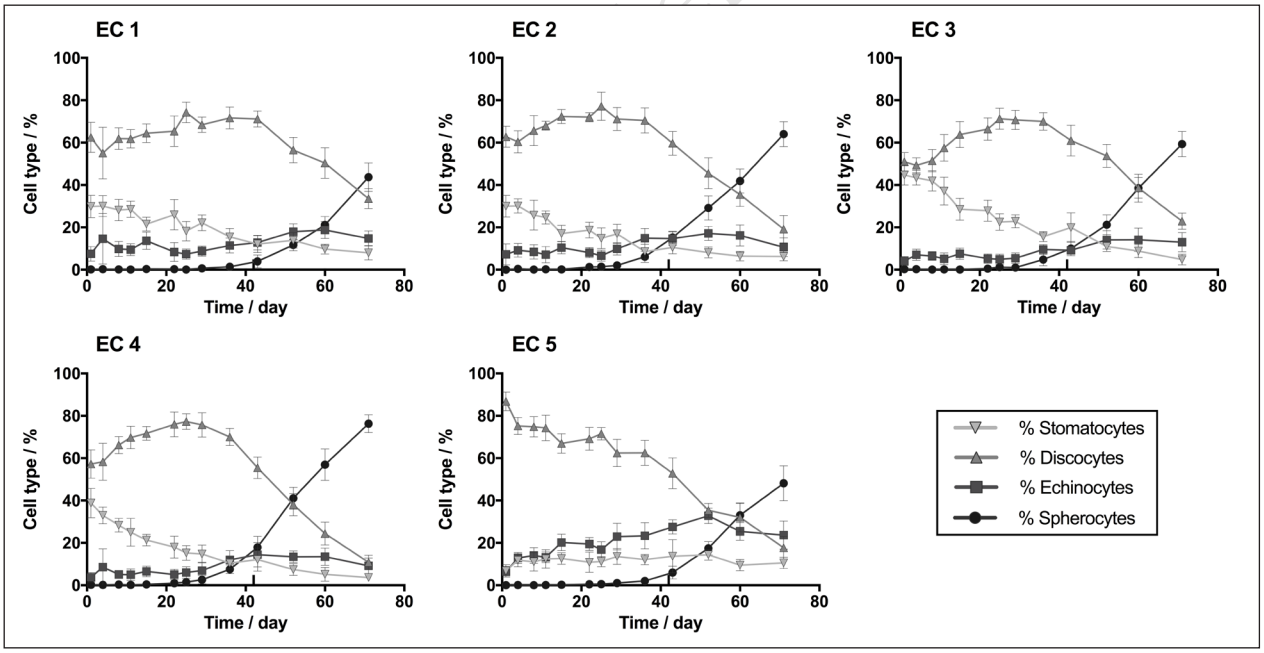

Figure S3 - DHM analysis of single-cell (CellProfiler and CPA) morphology of RBCs for ECs 1-5 stored during 71 days.
Twelve images (3 wells per EC and 4 images per well) and 3 movies (1 per well) were acquired for each EC. Mean values are presented ± standard deviation. CPA: CellProfiler Analyst; RBC: red blood cell; EC: erythrocyte concentrate.