Supplementary Information

Changes in RBC intracellular Calcium content over storage time

During blood storage, as adenosine 5 -triphosphate (ATP) decreases over time, an increase in RBC Ca^{2+} develops irreversibly.[1] Calcium influx is well known to promote eryptosis as well as microparticle formation, partially via phosphatidylserine externalization.[2-5]

In this work, we also investigated single RBC Ca^{2+} concentration in parallel to the deformability measurement (Figure S1 B). The intracellular Ca^{2+} level was found to increase steadily for the first 2 – 3 weeks of storage, and then remained fairly unchanged up to week 5. Scatter plots were also generated mapping out both Ca^{2+} and velocity (deformability) of individual RBCs from the same blood source on different storage days (Figure S1 B).

We also noted that the Ca^{2+} staining protocol may introduce additional stiffening effect on banked RBCs given the additional hours required for sample preparation and the exposure of RBCs to a buffer containing 1.8mM Ca^{2+} ions (i.e. Calcium influx). For that reason, we verified our deformability measurement using cell tracker orange dye (Figure S2 B). RBCs collected from the side outlet exhibited significant stiffening similar to that observed with Ca^{2+} staining.

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