VIII International School and Conference on Photonics, August 23 - August 27, 2021, Belgrade, Serbia
PHOTONICA 2021

Discovering abnormal erythrocyte membranes - optical approaches

Science Fund of the Republic of Serbia

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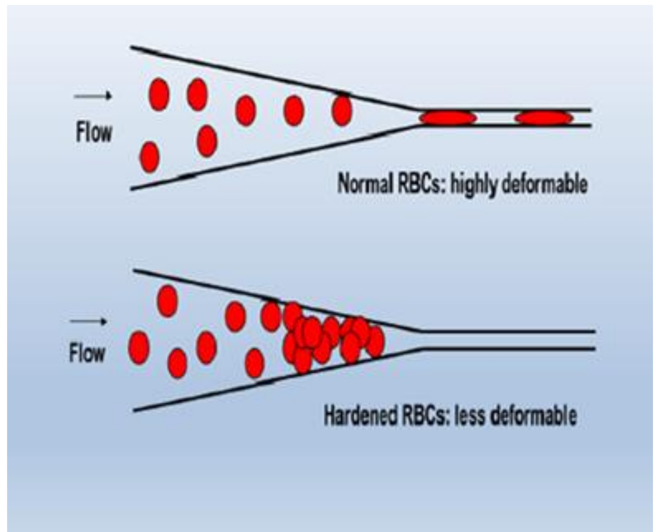
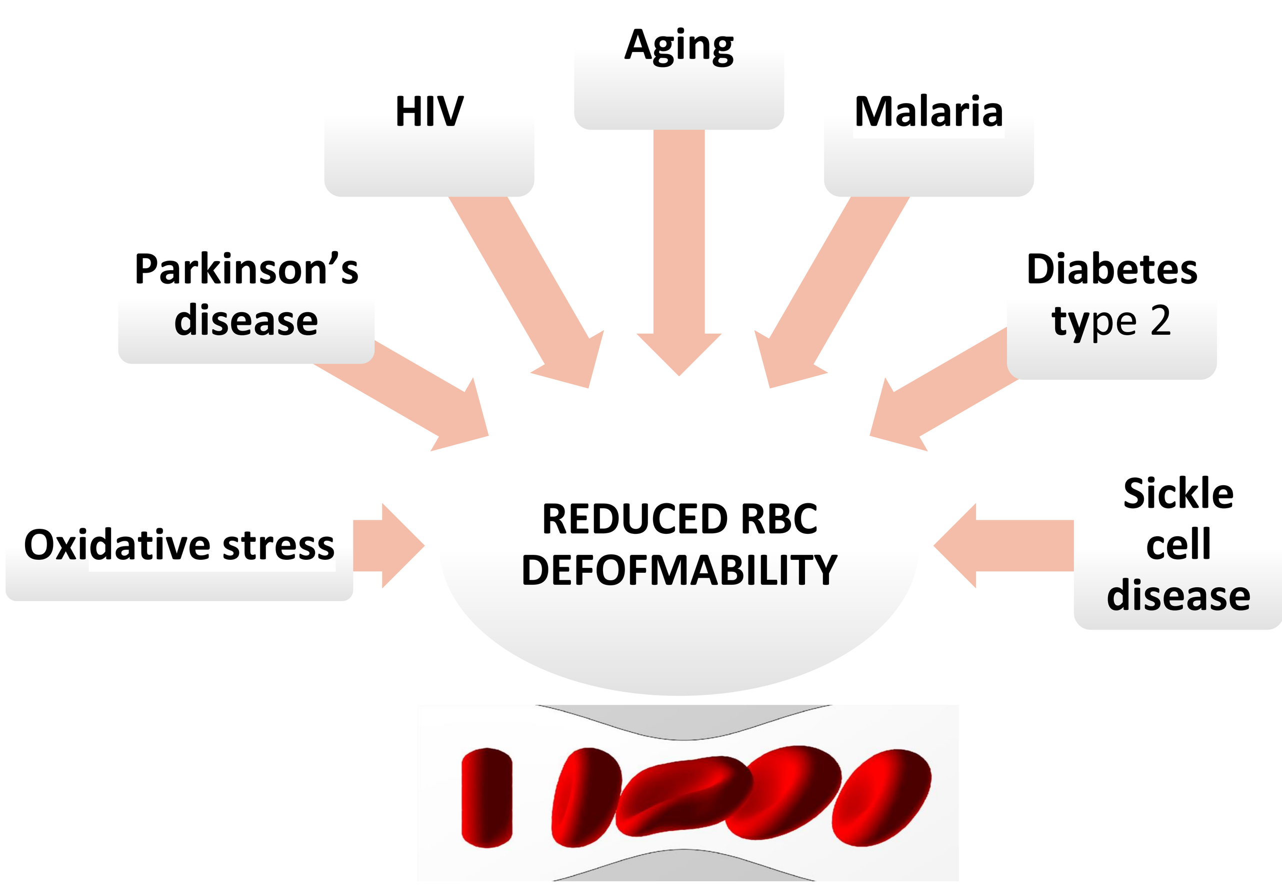
MOTIVATION

- Due to their complex physiological role, erythrocytes (RBC) have naturally very elastic membranes, are however, extremely susceptible to various endogenous and exogenous factors.



Schematic presentation of RBC deformability

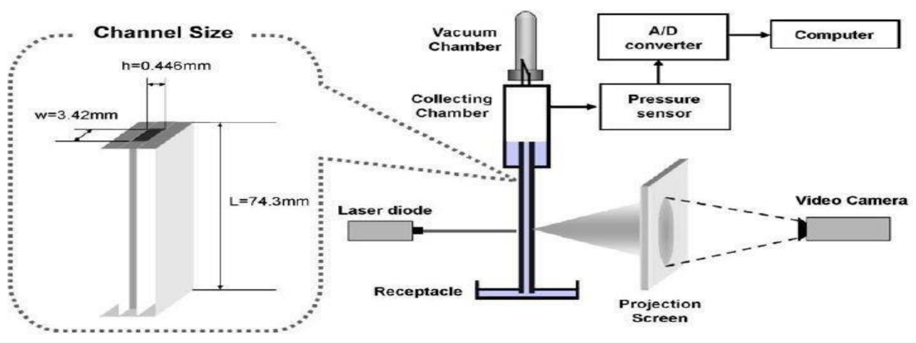
- It has been speculated that abnormalities in RBC membrane deformability and shape can be seen as an early sign of some acute and chronic pathological states/diseases [1,2].

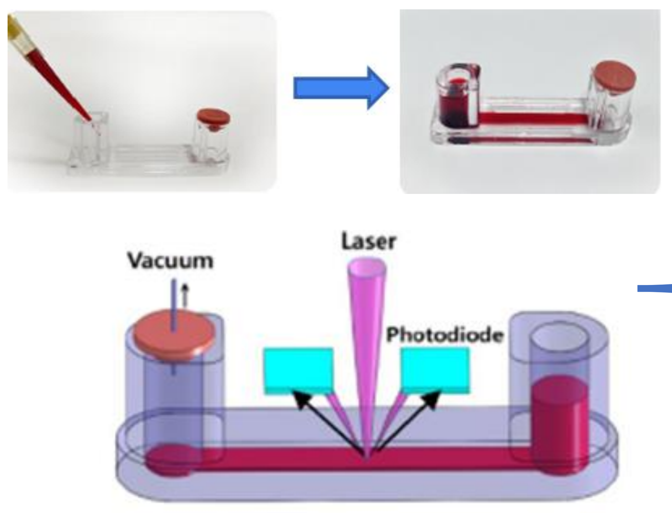


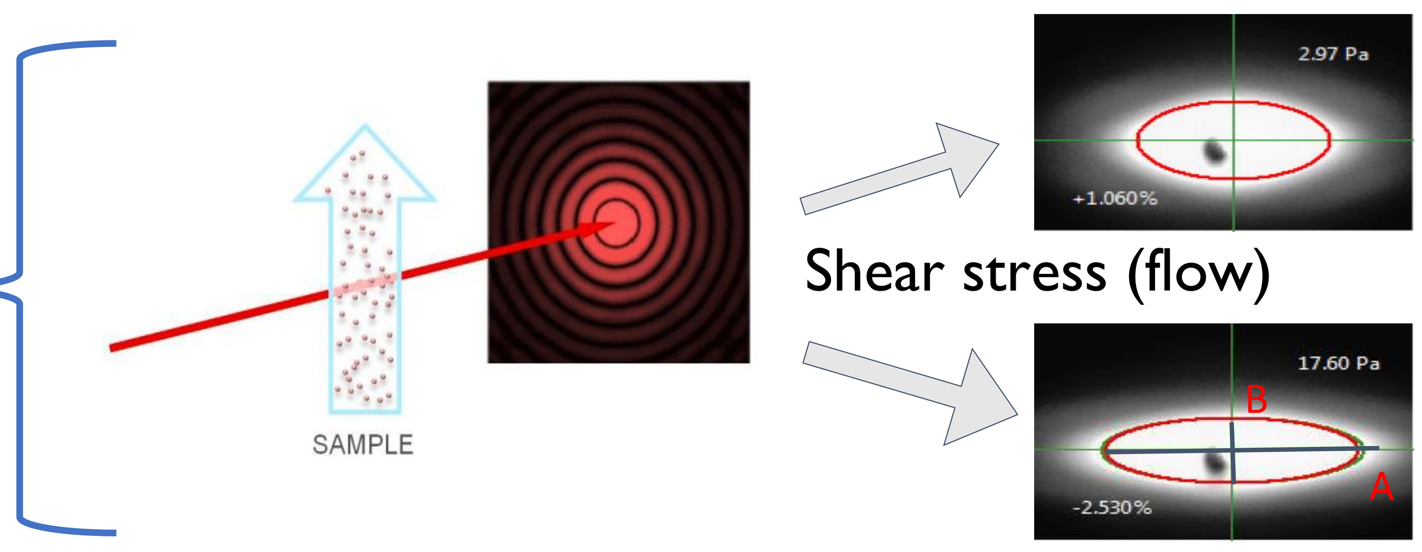
- In **HEMMAGINERO** project, we are exploring whether optical methods, ektacytometry, and Two-Photon Excitation Fluorescence microscopy, (TPEF) can be used as potential diagnostics tools in identifying any changes in the shape/deformability of erythrocytes.

MATERIALS AND METHODS

Ektacytometer RheoScan D300





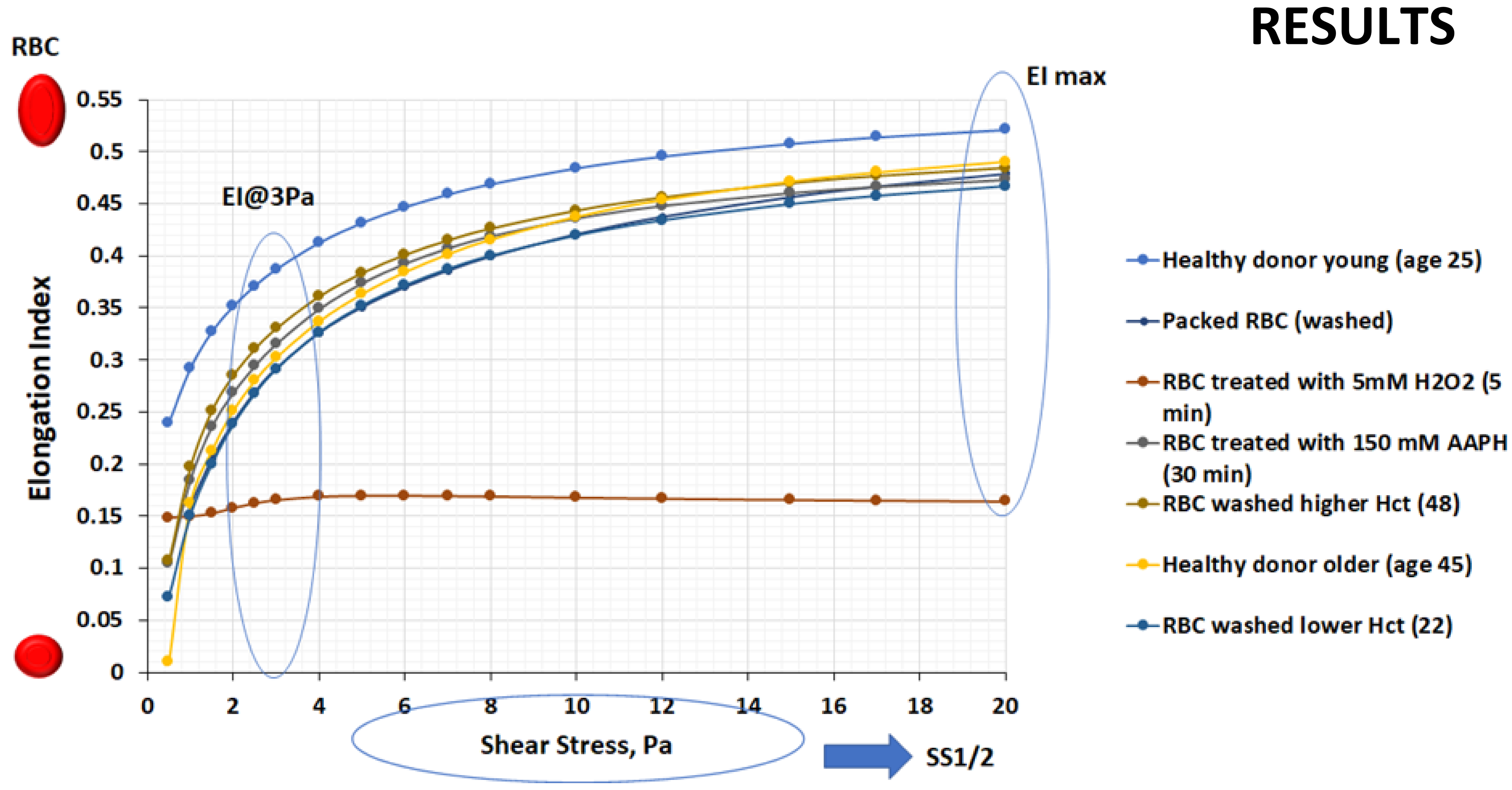


$$EI = \frac{A-B}{A+B}$$

A-major axis
B-minor axis

- Result is given through **Elongation index (EI)**
- **EI(max)- the maximal RBC elongation index**
- **SS(1/2) - the shear stress required for one-half of this maximal deformation**
- **EI@3Pa-used for inter-lab comparison (3Pa is an average pressure in microvasculature)**

RESULTS

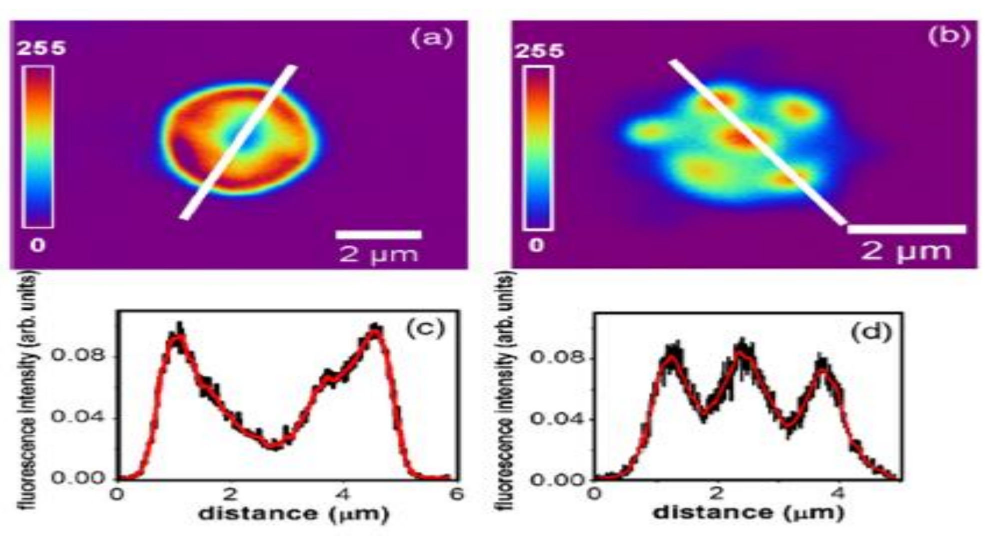


Deformability curves of healthy donors, isolated and washed RBC and RBC after treatment with oxidative agents (one sample was treated with hydrogen peroxide (H_2O_2) other one with AAPH

Sample	Elmax	SS1/2	EI@3Pa
Healthy donor young (age 25)	0.589	2.002	0.387
Healthy donor older (age 45)	0.545	2.474	0.302
Packed RBC (washed)	0.524	2.439	0.292
RBC washed higher Hct (48)	0.535	2.002	0.330
RBC washed lower Hct (22)	0.517	2.317	0.291
RBC treated with AAPH 150 mM	0.473	2.003	0.315
RBC treated with H_2O_2 5mM	0.301	5.539	0.164

- The older healthy donor vs the younger: SS ½ ↑ elongation indexes ↓
  This implies greater rigidity of the membrane (presumably because of oxidative stress due to aging process).
- RBC treated with H_2O_2 5mM vs control sample (Packed RBC washed) SS ½ ↑↑ elongation indexes ↓↓
  Very rigid membranes due to oxidative stress- *in vitro*

We have already demonstrated by in-house developed TPEF microscopy set-up that hemoglobin distribution follows RBC morphology [3], which is related to RBC deformability.



Raw TPEF image of (a) human and (b) porcine erythrocyte (pseudo color), hemoglobin concentration through the diameter of (c) human and (d) porcine erythrocyte.

Both ektacytometry and TPEF microscopy are sensitive and reliable in determining that membranes of erythrocytes have suffered under non-ideal (meaning non-physiological) conditions of the *in vitro* environment.

Further investigation is needed to conclude the precision of these optics methods in discovering abnormal erythrocyte membranes in actual patients' blood.

REFERENCES:
[1] H. Chen et al. Clin. Hemorheol. Microcirc. 16, 2 (1996).
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[3] K.S. Bukara et al. J. Biomed. Optics 22(2), 026003 (2017).

Funding Project: HEMMAGINERO