Pulsed field ablation induces hemolysis in vitro depending on the electric field strenght

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Pulsed field ablation (PFA) for atrial fibrillation

used **next to RFA** for **PVI isolation**

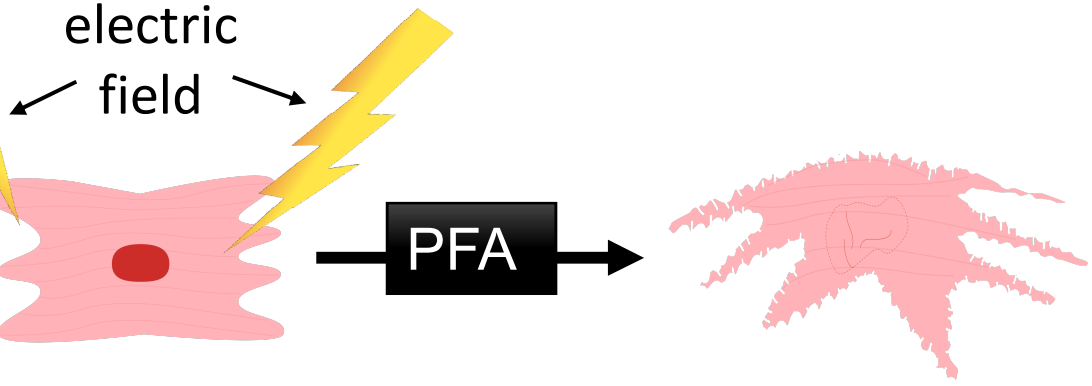
More **effective, less sides effect, faster** intervention process

Irreversible **pore formation** in the cell membrane due to **high voltage electric pulses** → **cell death** (Ca^{2+} overload?)

Possible sides effect:

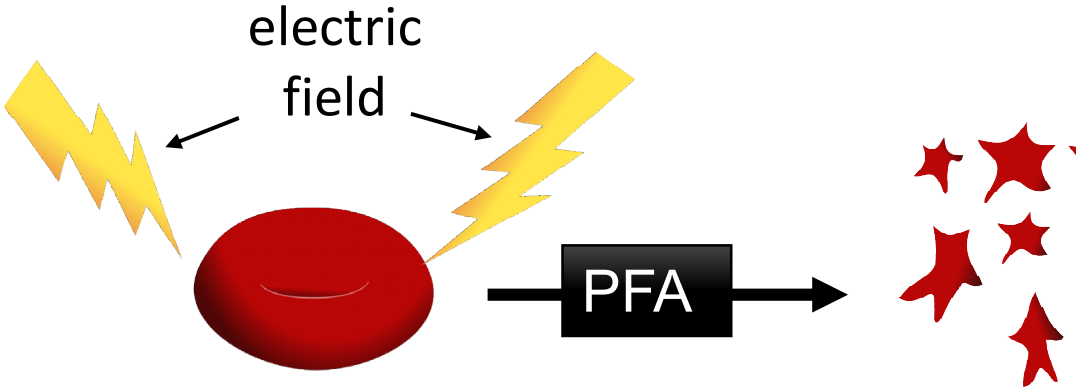
renal failure (rare cases)

intravascular hemolysis (all patients)



cardiomyocyte

cardiomyocyte death



Red blood cell (RBC)

hemoly

Hypothesis

High voltage electric pulses induce **damage of red blood cells** depending **on the electric field strength** *in vitro.*

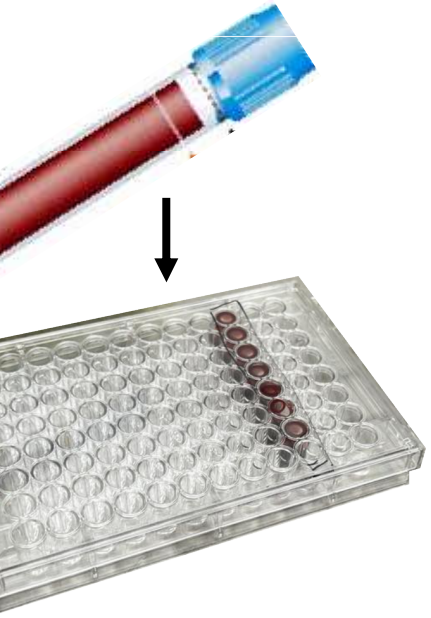
Aim

To determine the extent of **red blood cell damage** with **increasing electric field strength** *in vitro*.

Methods

xperimental model

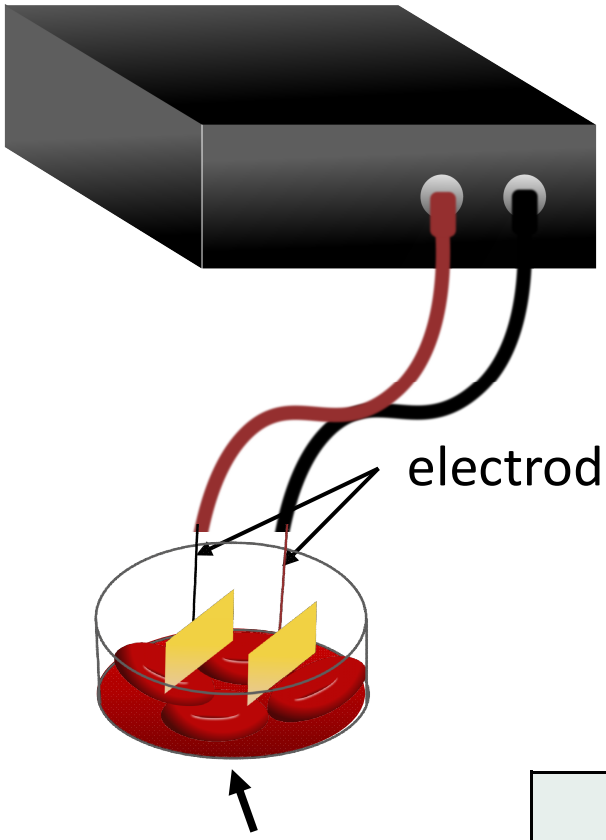
lood samples from **healthy volunteers.**



vell microplate

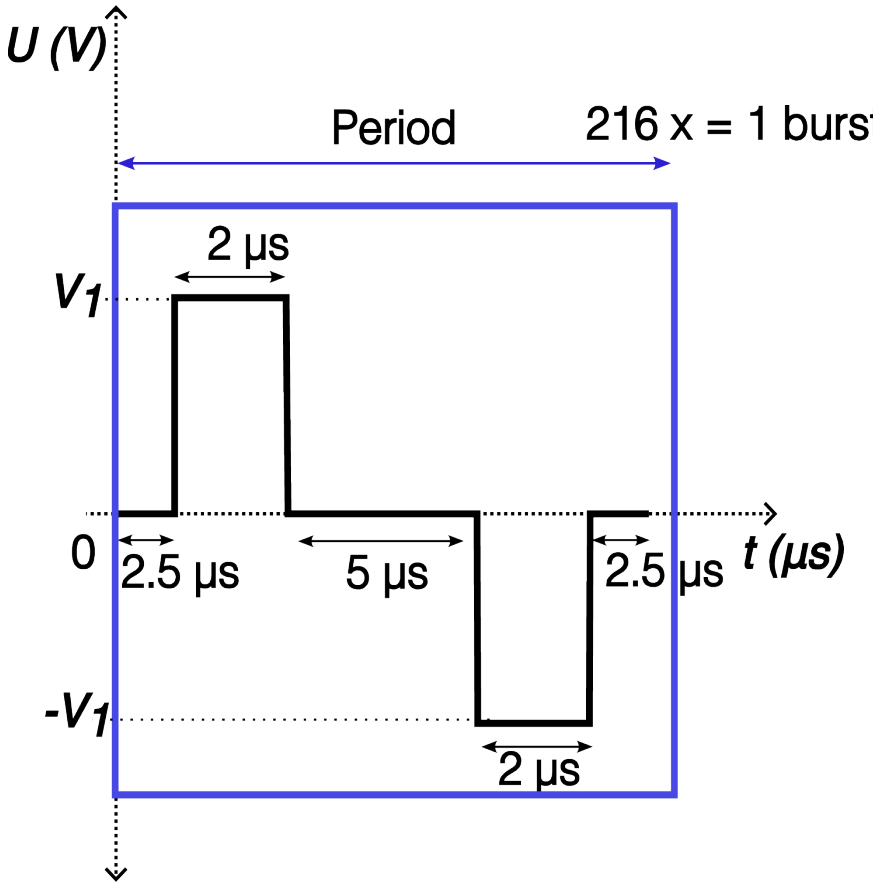
Electroporation setup

Electric pulses generator



electrodes

Blood in cell culture well



$$\overrightarrow{E} \approx (3 \cdot U) + 20\,\%$$

Voltage (V)	0	90	190	290	380	480	5
Electric field (V/cm)	0	250	500	750	1000	1250	15

Methods

Sample collection

od samples - centrifuged for plasma separation

Cell free hemoglobine

Harboe direct spectrophotometric method

$$\left(g/l\right) = \frac{k * (167.2 * A_{415} - 83.6 * A_{380} - 83.6 * A_{450})}{1000}$$

= absorbance

$_{15}$ = absorbance of oxyhemoglobin

$_{80}$ = absorbance of non-specific plasma

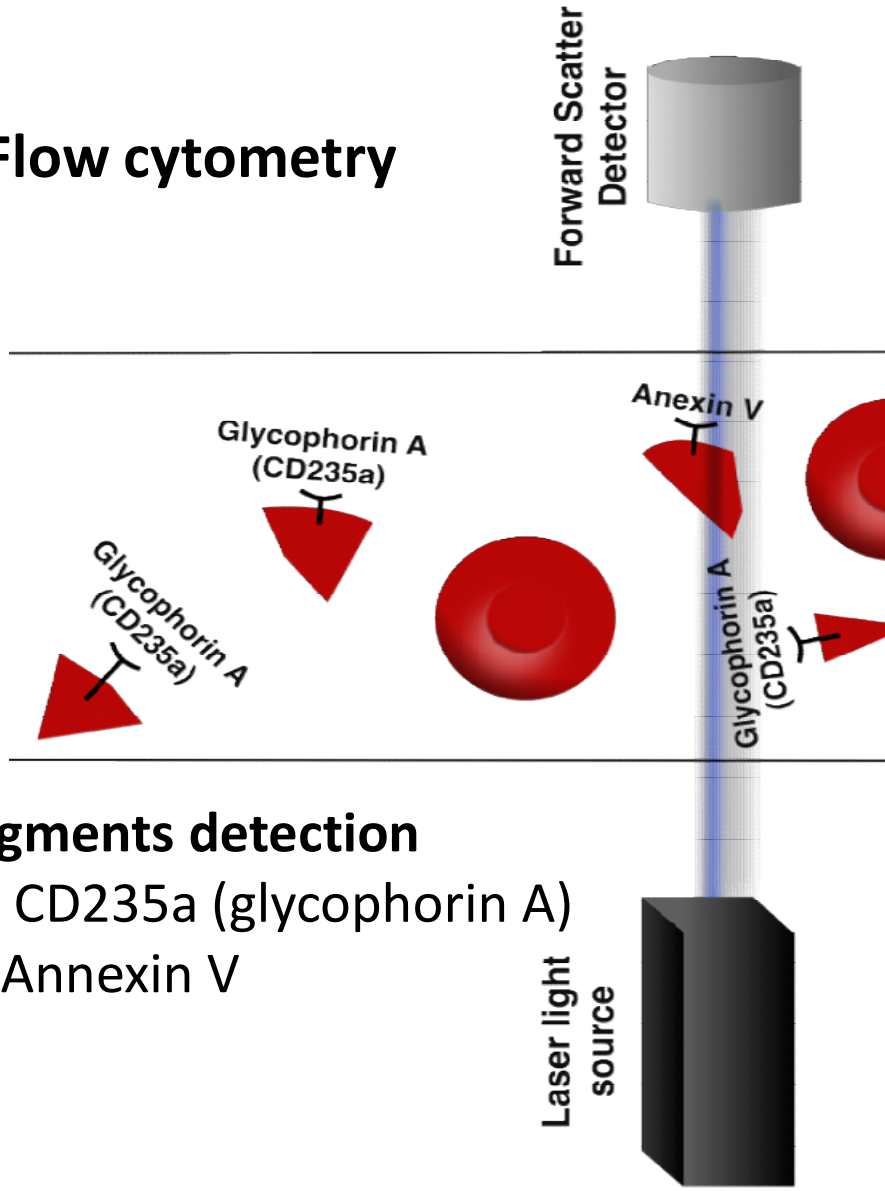
erferents

$_{50}$ = bilirubin/albumin complexes

dillution

Red blood cells microparticl

Flow cytometry

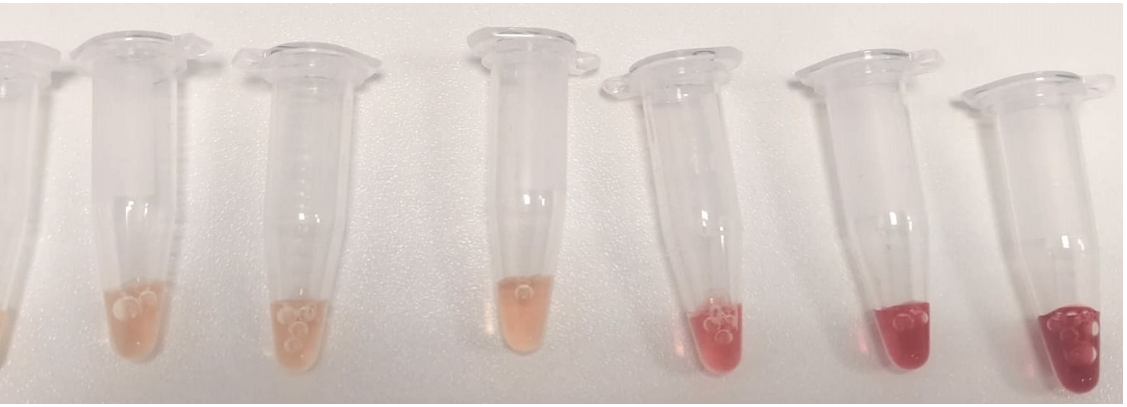


fragments detection

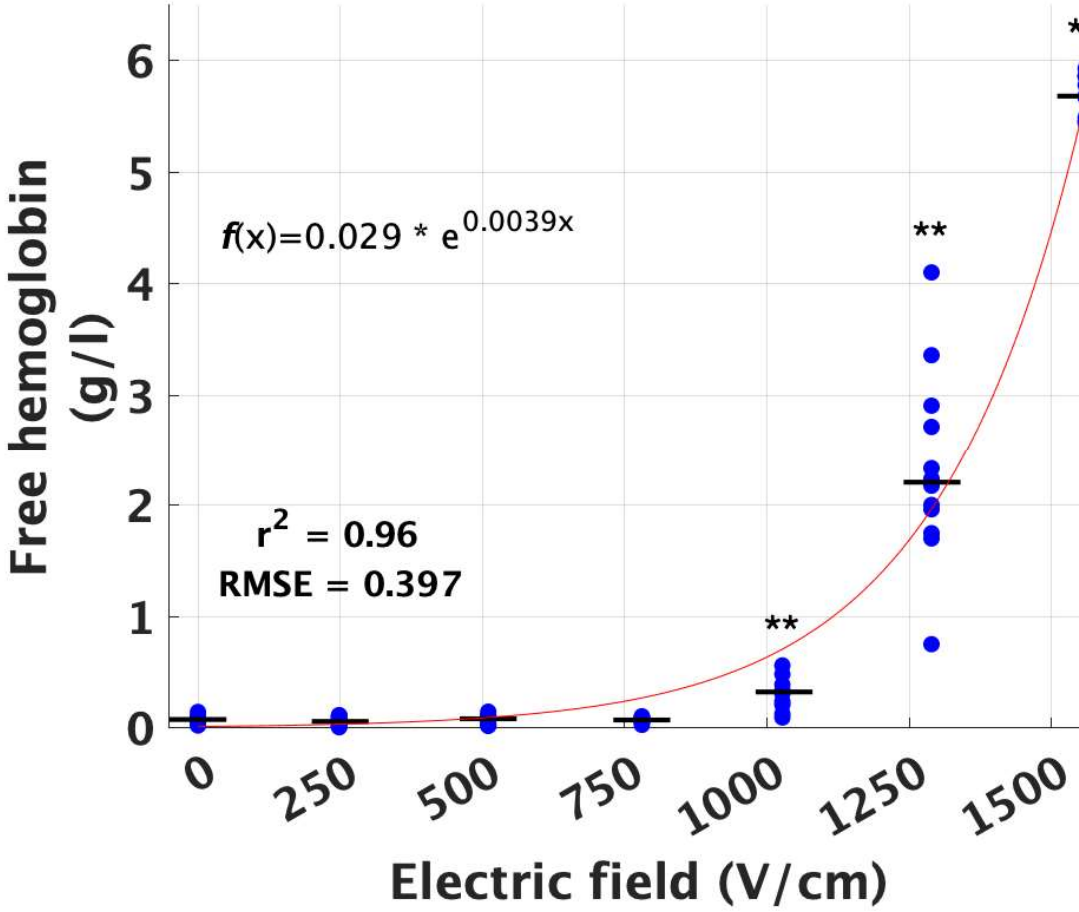
- CD235a (glycophorin A)
- Annexin V

esults

Separated plasma samples after in vitro PFA (examples)



cm | 250 V/cm | 500 V/cm | 750 V/cm | 1000 V/cm | 1250 V/cm | 1500 V/cm



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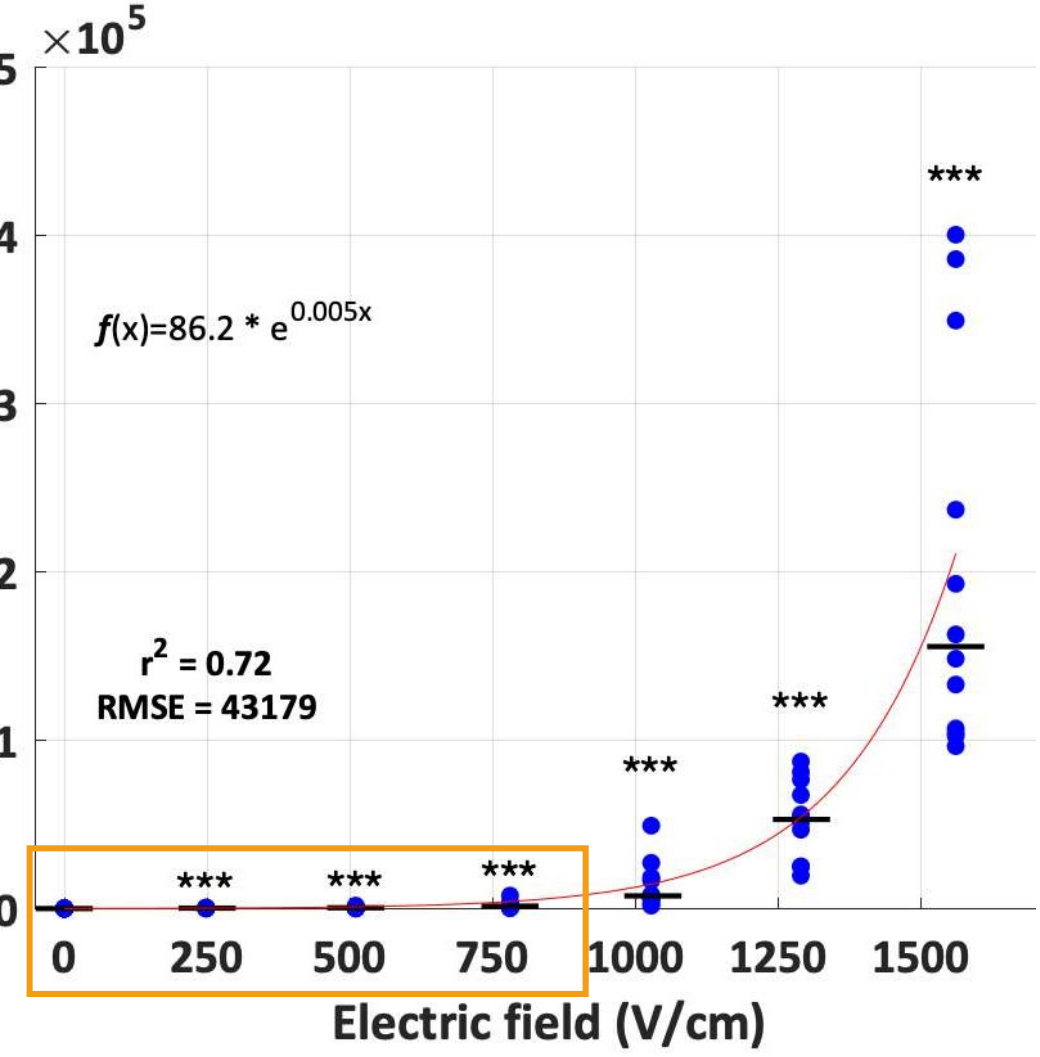
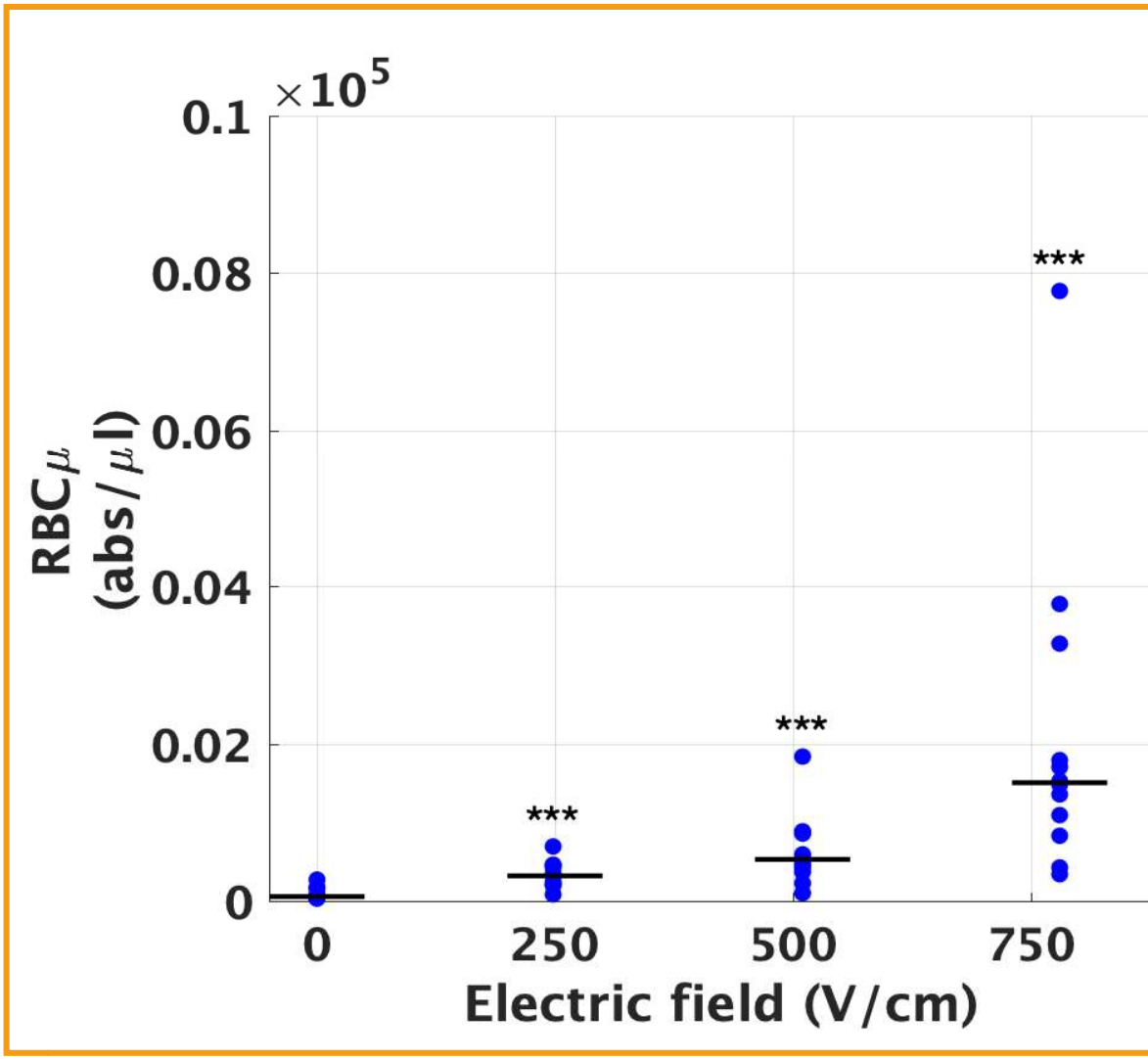
esults

Red blood cells microparticles





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onclusion

- From **1000 V/cm - free hemoglobin released** from RBC to **plasma**
- **↑ electric field → ↑ the breakdown of RBC at all electric field strengths**

RBC are damaged by high voltage electric pulses

uture perspectives

- to investigate the **influence of the cardiac cycle**
- to find an **ablation protocol with minimal hemolytic effect** and **preservation** of effective **cardiomyocyte death**

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Thank you for your attention!

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