Electroporation of cardiomyocytes in vitro

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Electroporation

= formation of **pores** in the **cell membrane by short high-voltage pulses**

According to the electric field setting, length and number of pulses

Reversible

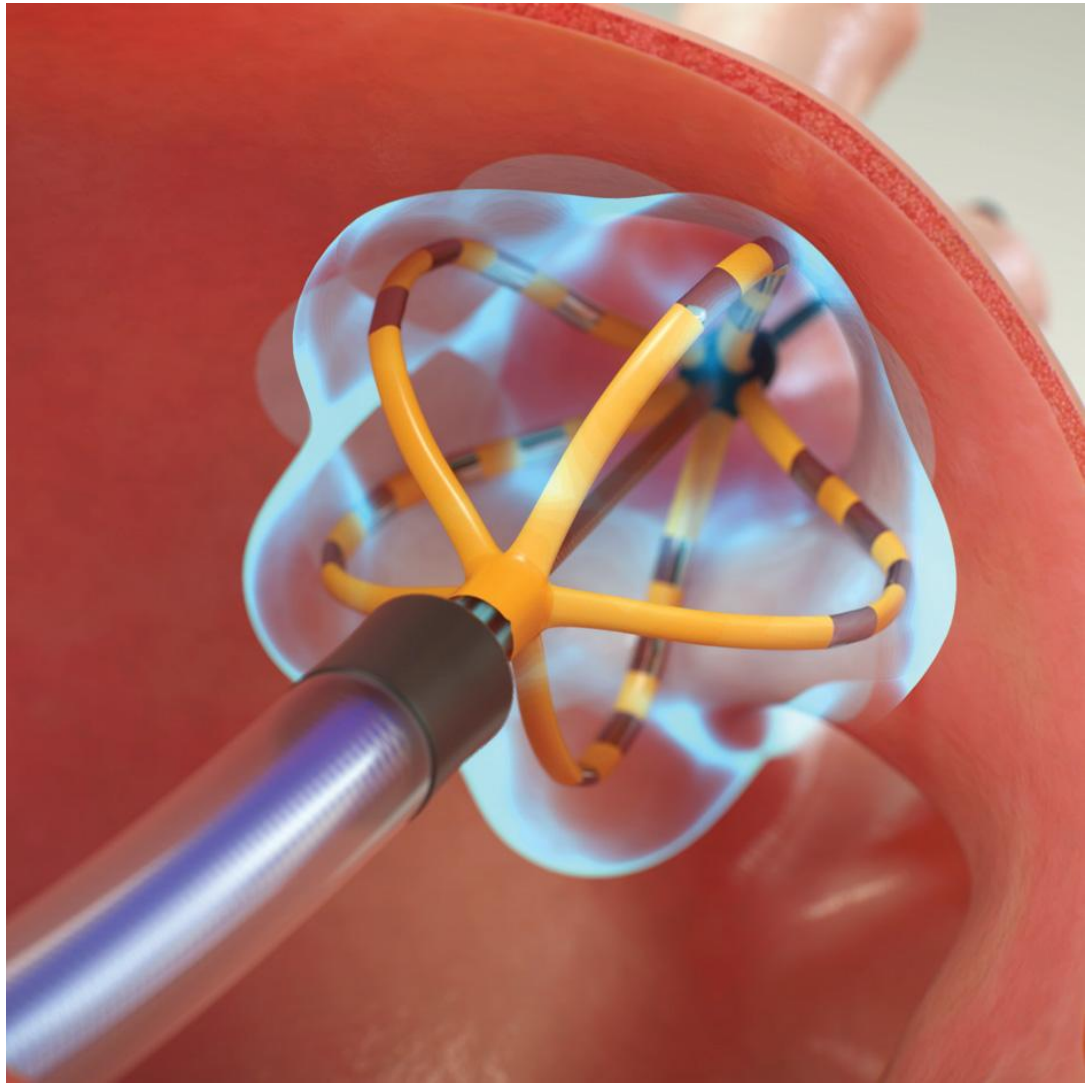
- **temporary** increase in cell membrane permeability through pores
- **transport of large molecules** (DNA, RNA, chemotherapeutics) → **gene transfection**
- **lower** electric field values

Irreversible

- **permanent** increase in cell membrane permeability
- induction of cell **death in ectopic lesion**
- **higher** electric field values

Irreversible electroporation = pulsed field ablation

- **Cathetrisation therapy for atrial fibrillation**
- Cardiomyocyte **death** due to **pores formation** and **Ca^{2+} overload (???)**
- **Faster** intervention process
- More **effective, less sides effects** (compare to RF ablation)



FARAPULSE - catheter for the treatment of atrial fibrillation

Hypothesis

A characteristic threshold adjustment of the electric field intensity, number and length of pulses can **induce irreversible electroporation** of **cardiomyocytes in vitro** without subsequent cell reparation.

Aim

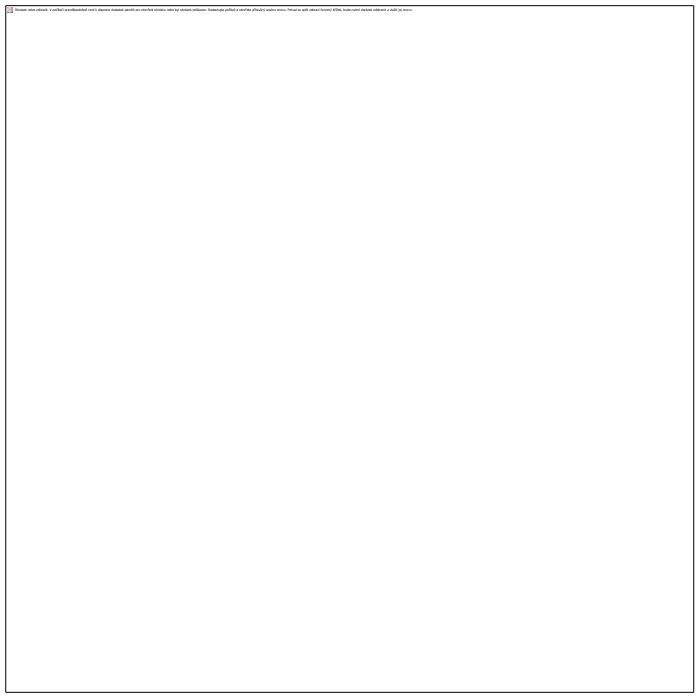
To find the electric field strength limit for irreversible electroporation of cardiomyocytes *in vitro*.

Methods

TONA|GENA

Experimental model

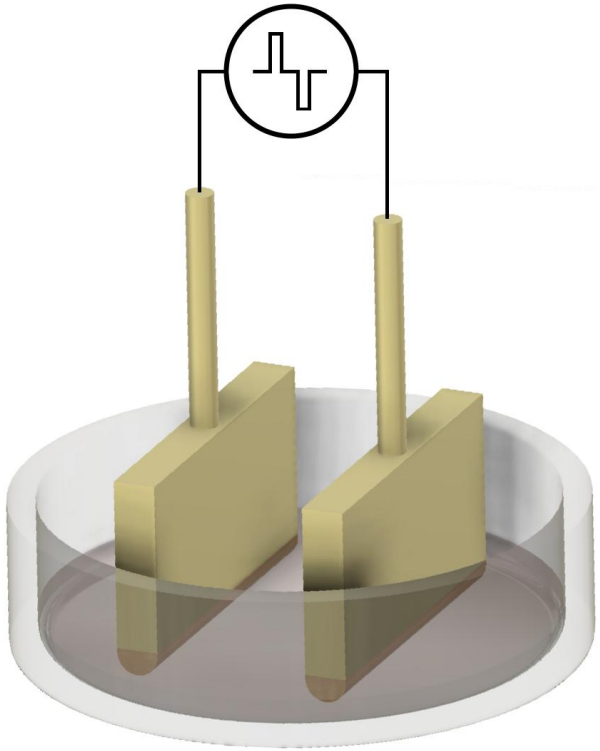
Immortalized **murine atrial cardiomyocyte** tumor line (HL-1).



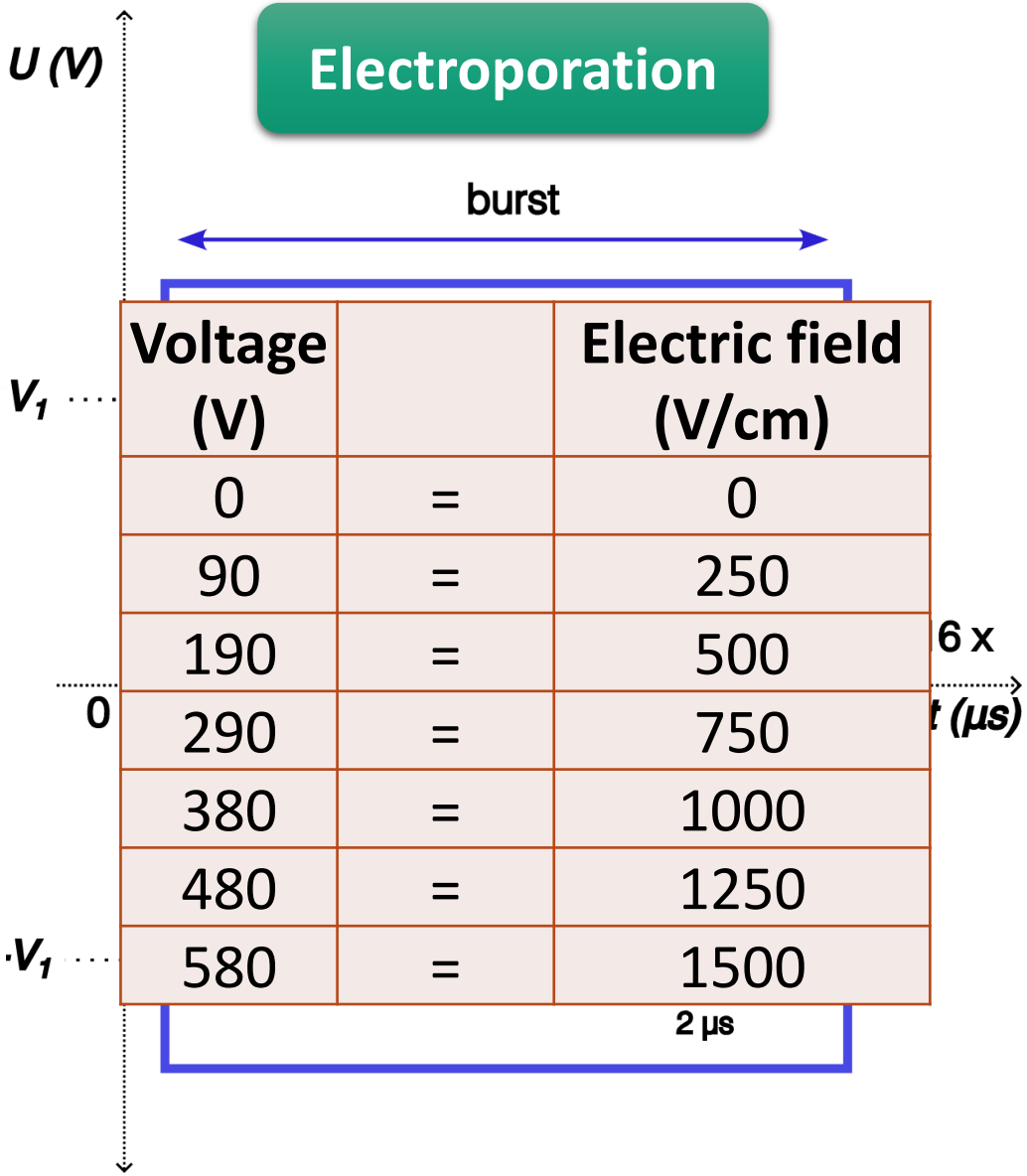
Electroporation

El. pulse generator with electrodes for 96-well plates.

Methods



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

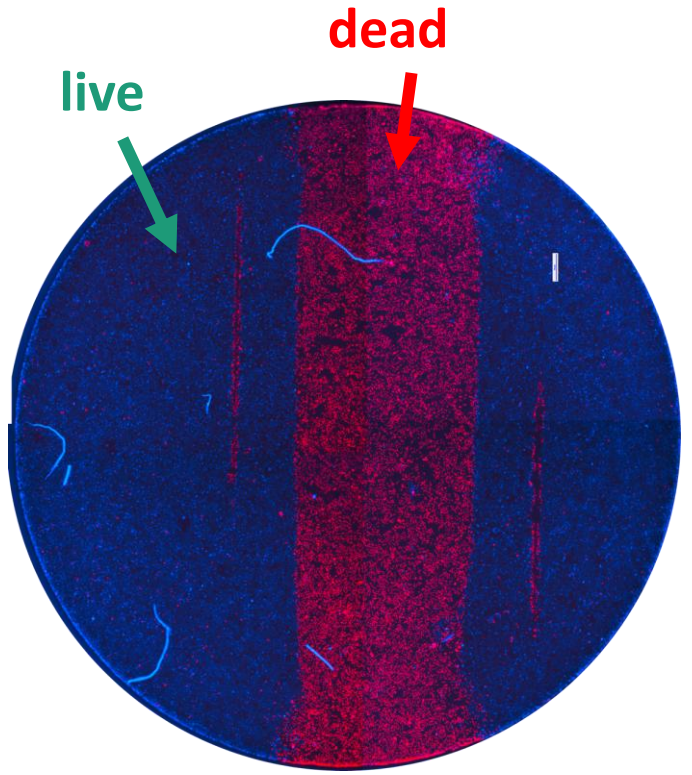


Voltage (V)		Electric field (V/cm)
0	=	0
90	=	250
190	=	500
290	=	750
380	=	1000
480	=	1250
580	=	1500

Electroporation

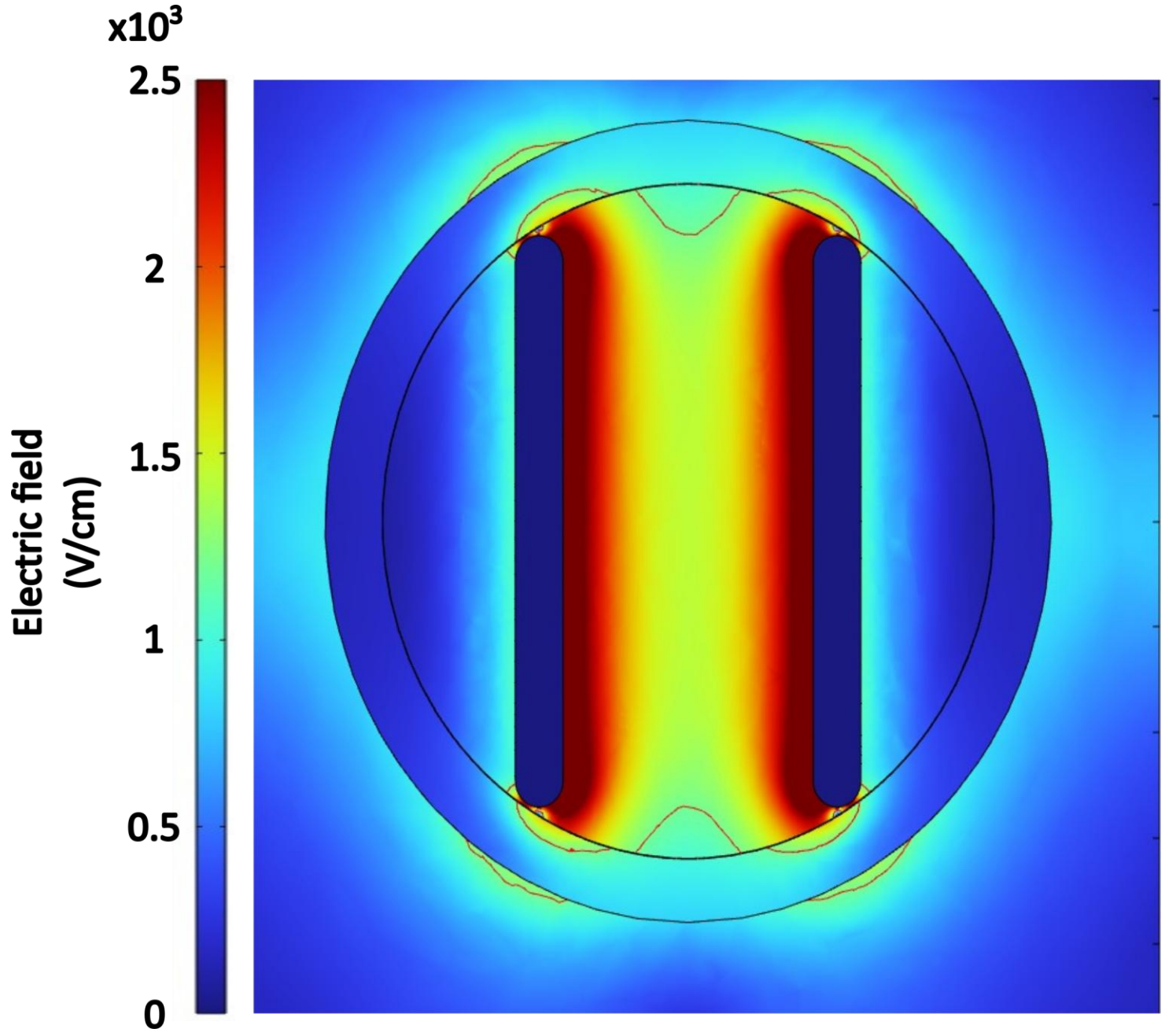
Detection of cell death

- Fluorescence microscopy
- 1 hour, 24 hours



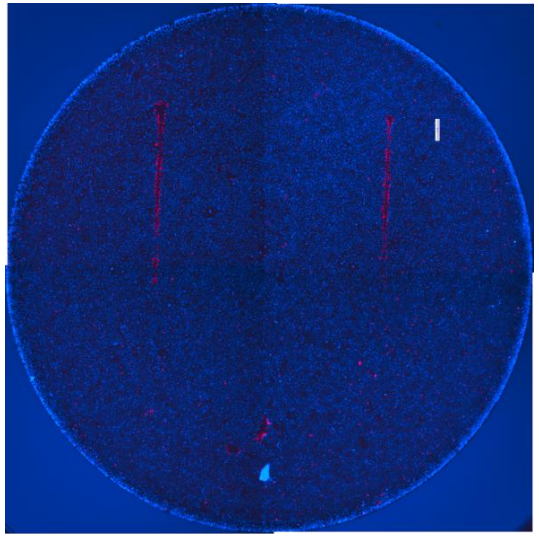
Results

Simulation of the electric field between electrodes (Comsol Multiphysics)



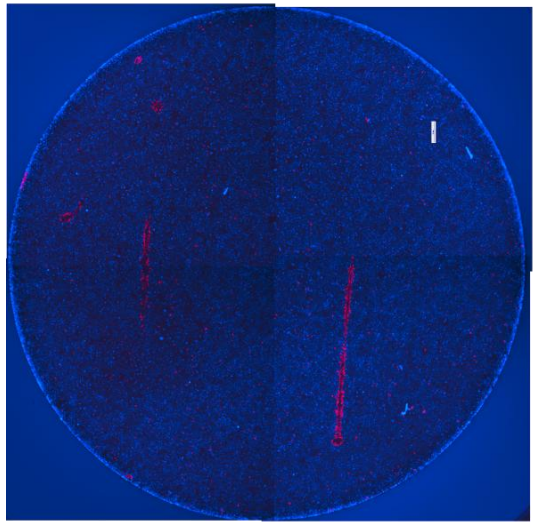
Results

control

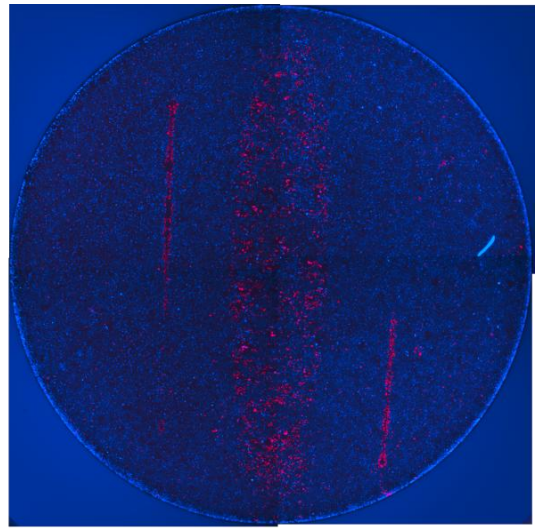


0 V/cm

Potencial reversible

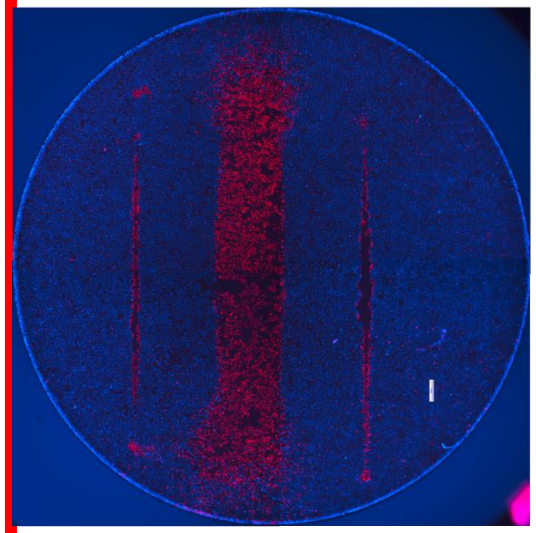


250 V/cm

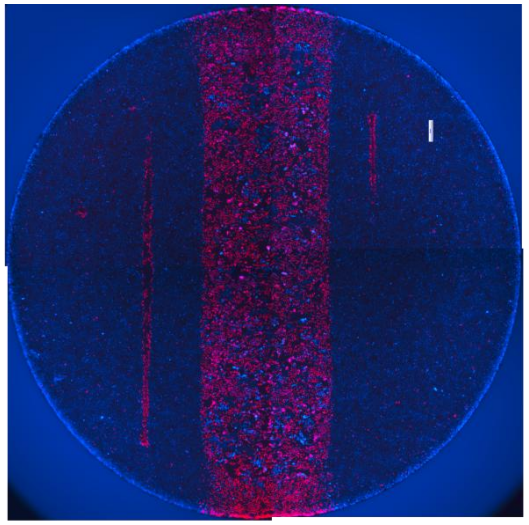


500 V/cm

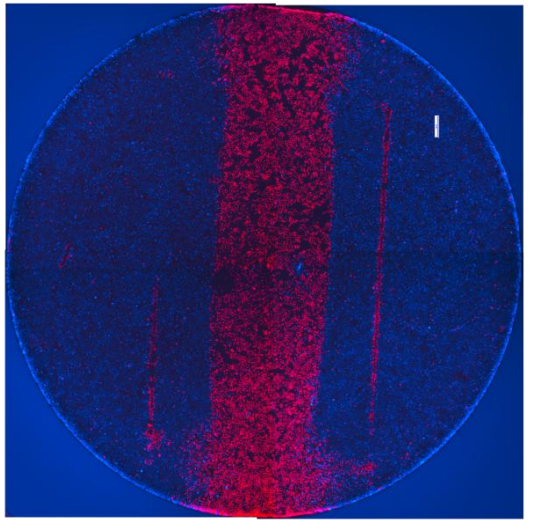
Irreversible



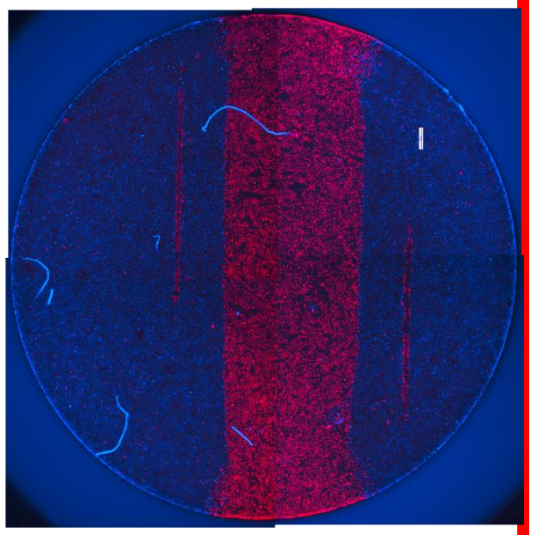
750 V/cm



1000 V/cm

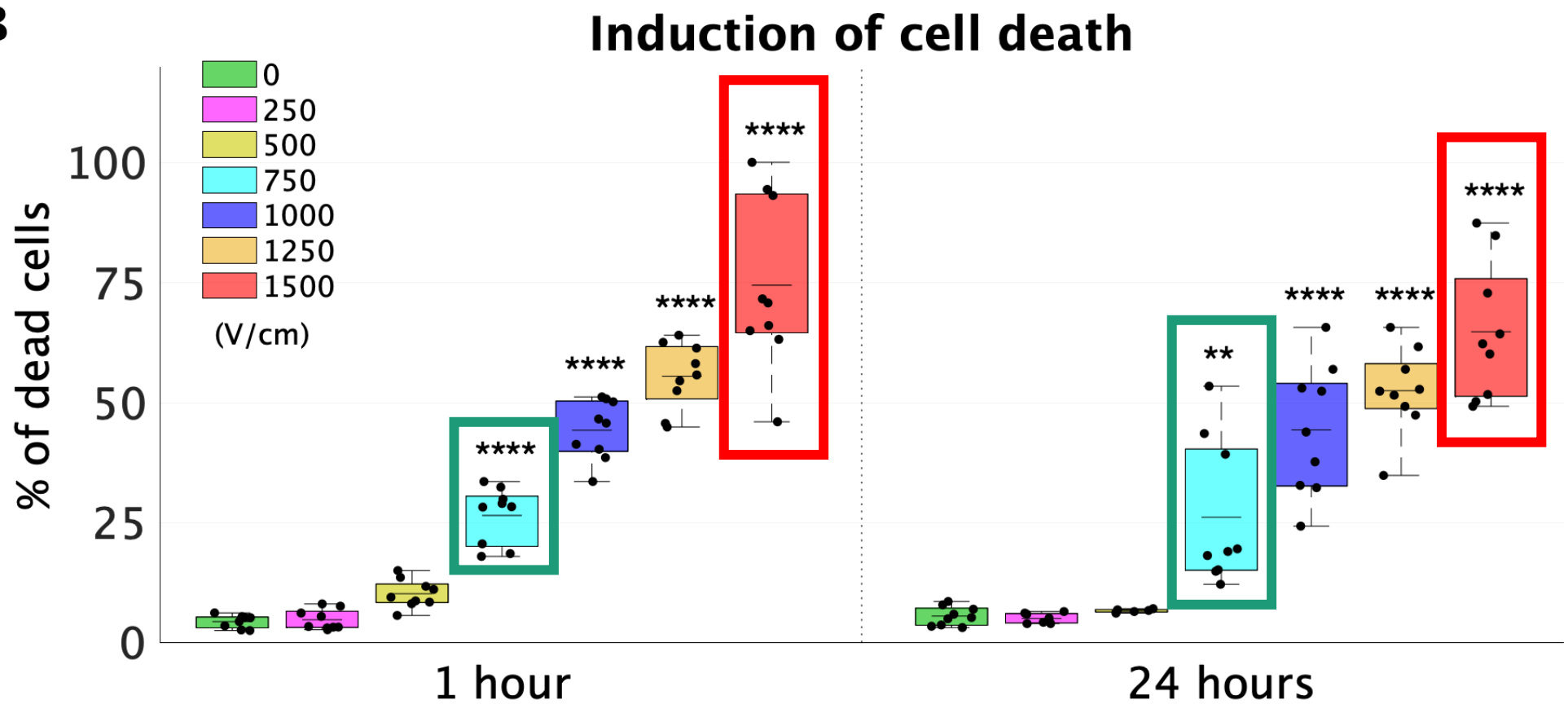


1250 V/cm



1500 V/cm

B



	0 V/cm	250 V/cm	500 V/cm	750 V/cm	1000 V/cm	1250 V/cm	1500 V/cm
1 h	5.2 ± 0.6	5.6 ± 2.0	11.1 ± 2.8	24.5 ± 6.2	43.8 ± 6.8	55.5 ± 8.6	**78 ± 21.4**
24 h	5 ± 2	4.3 ± 0.5	6.7 ± 1.4	16.5 ± 2.9	37.3 ± 10.0	50.3 ± 2.4	**62.3 ± 15.3**

Conclusion

- Electric field **up to 500 V/cm did not induce cell death** (suitable for gene transfection?)
- **Pulsed field ablation effective** at electric field strengths **above 750 V/cm**
- Highest efficiency achieved at **1500 V/cm (78 % death cells)**
- Cell death rate **similar** even in **24 hours**

Future perspectives

- **Gene transfection** at lower electric field strengths
- Recognition of the type of cell death – **necrosis x apoptosis**
- **Comparison of results with patients after pulsed field ablation**

Thank you for your attention!

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