Nanofibre Mats for Neurosurgery

Tomasz A. Kowalewski[1],
T. Kowalczyk[1], P. Nakielski[1],
M. Frontczak-Baniewicz[2], D. A. Gołąbek-Sulejczak[2], J. Andrychowski[2]

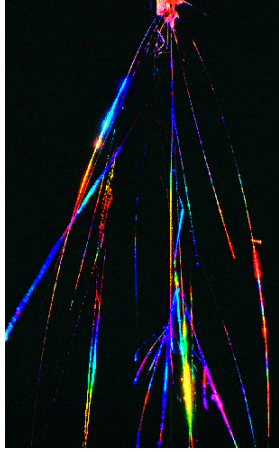
[1]*Institute of Fundamental Technological Research, Polish Academy of Sciences*

[2]*Mossakowski Medical Research Centre, Polish Academy of Sciences*


IMDiK
POLSKA AKADEMIA NAUK
IPPT
PAN
Biocentrum
ochota

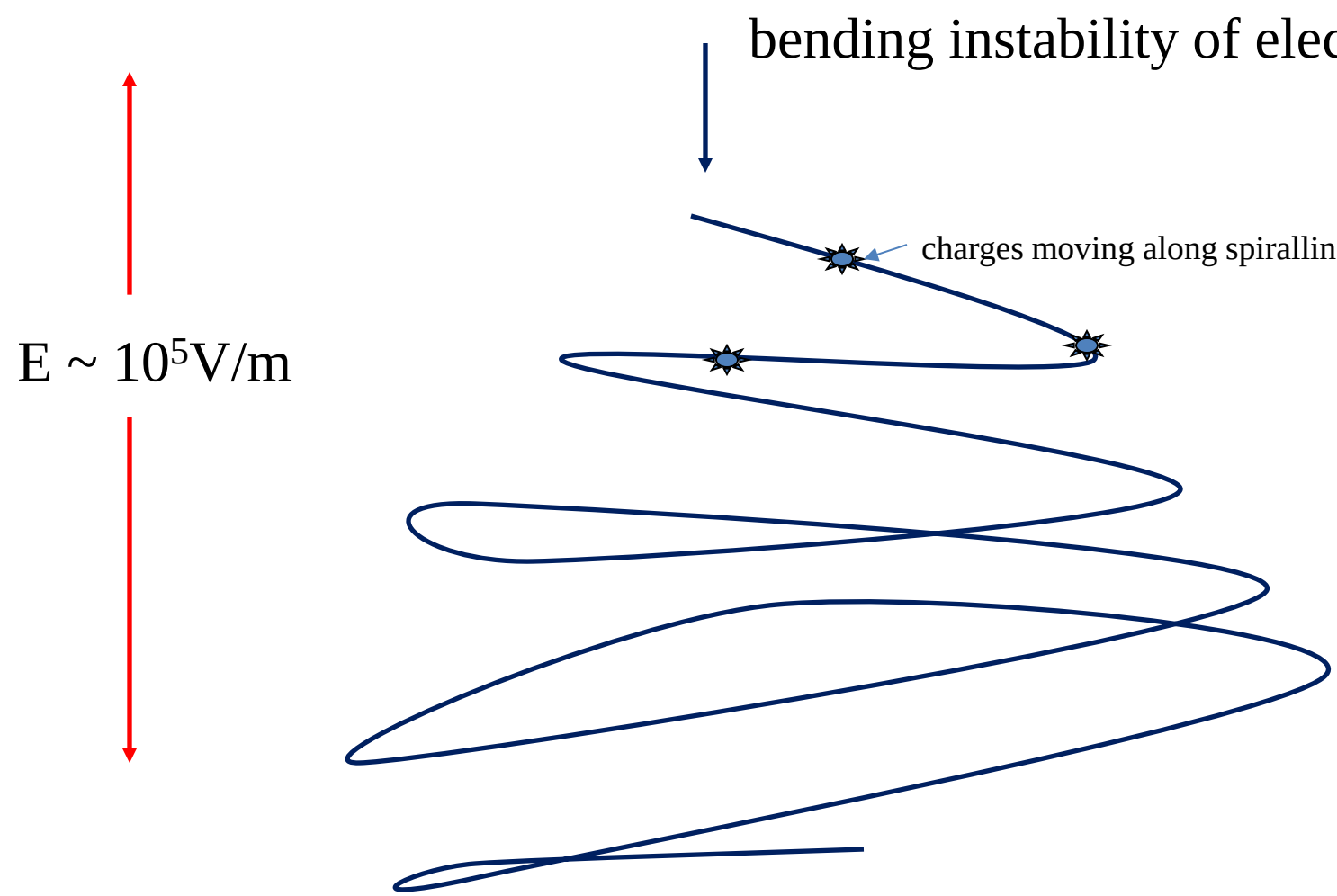
Electrospinning of

NANOFIBRES



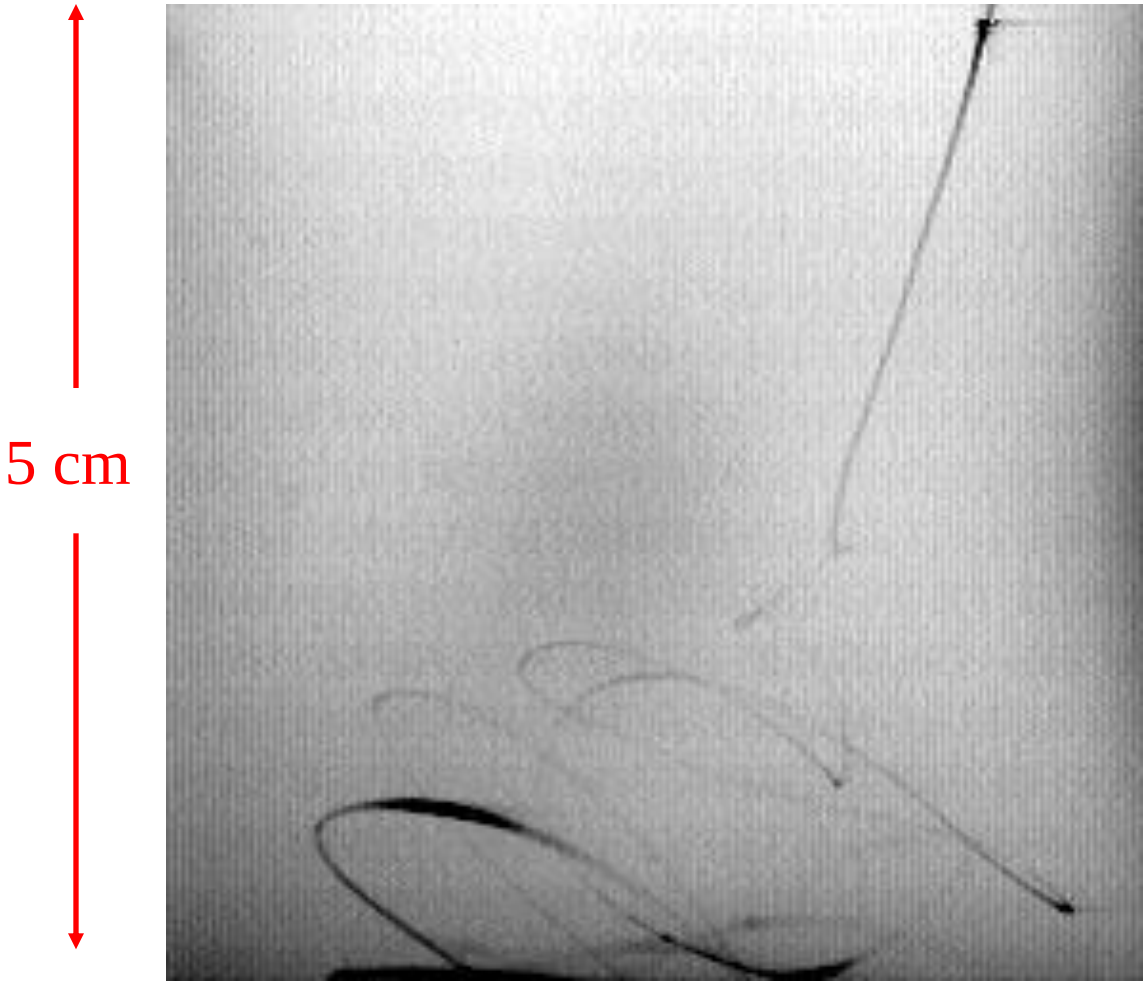
- **Reduction of characteristic dimension -> nano-biotechnology, tissue engineering, drug delivery**
- **Bio-active fibres: catalysis of tissue cells growth**
- **Mechanical properties improvement -> new materials, composite materials, co-fibres of metal-polymer, nano tubes**

Electro-spinning



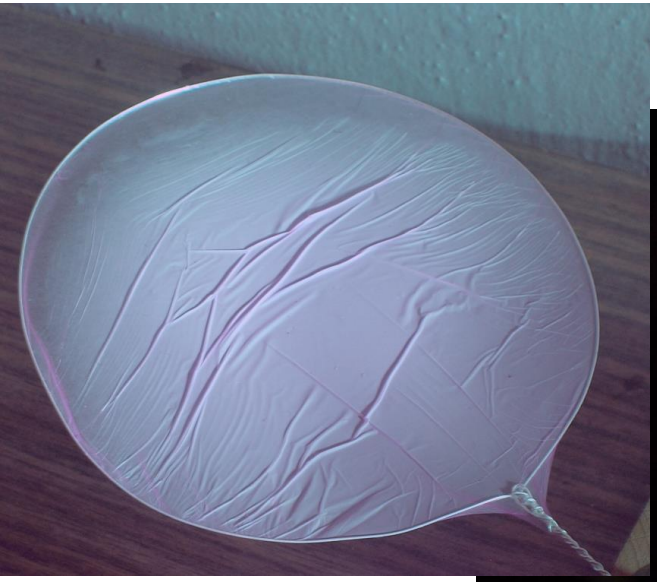
Bending instability enormously increases path of the jet, allowing to solve problem: how to decrease jet diameter 1000 times or more without increasing distance to tenths of kilometres

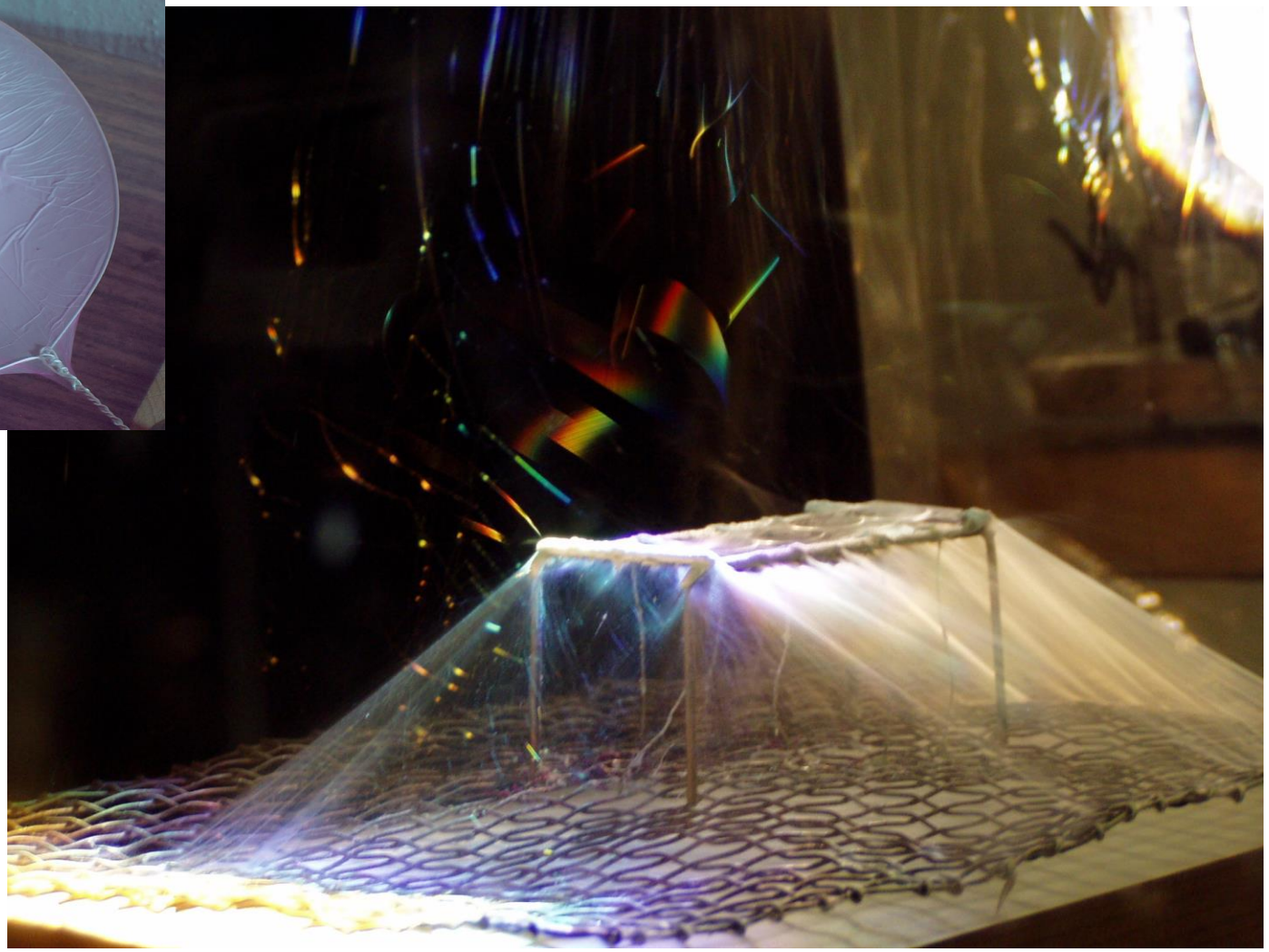
Electrospinning observed at 4500fps

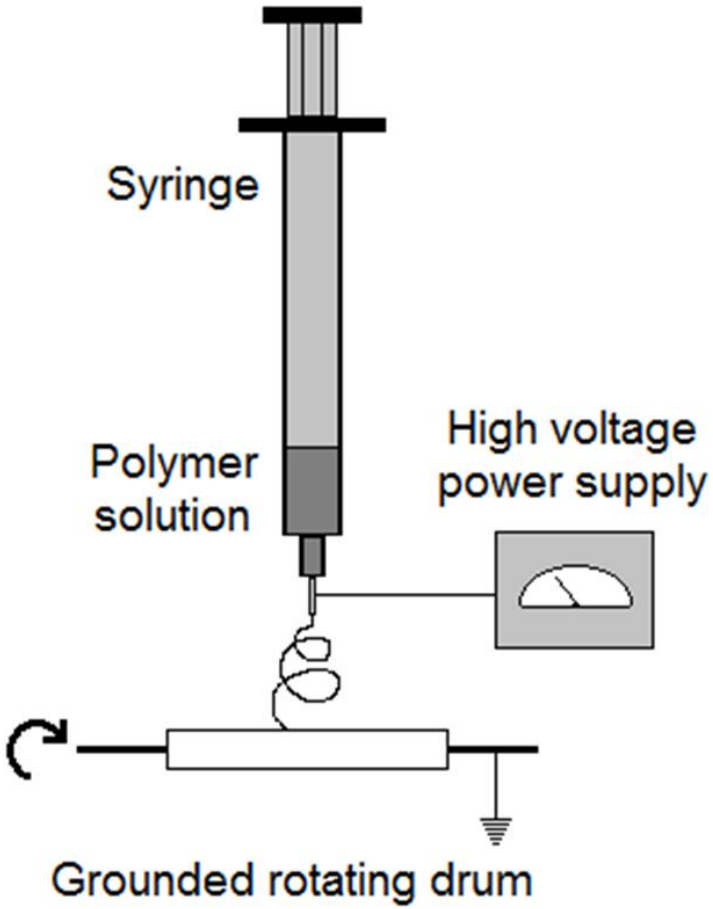


PEO
(poly ethylene oxide)
+ water/ethanol

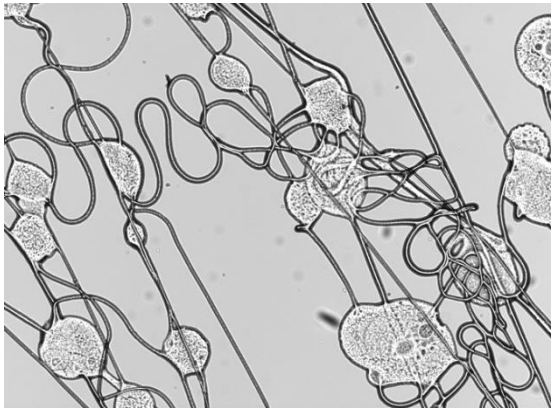
Nanofibres collection

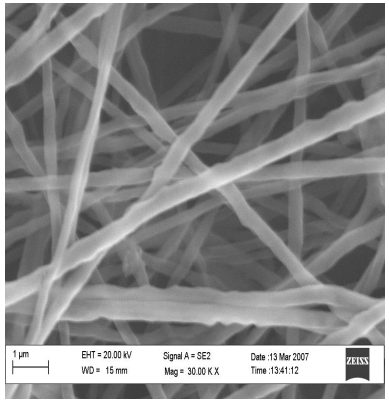


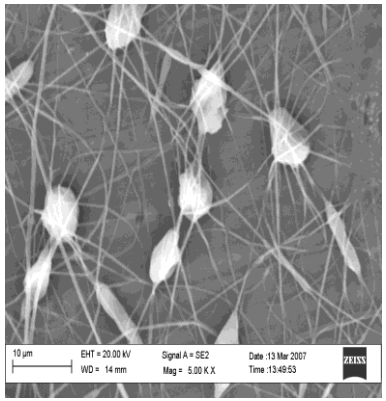


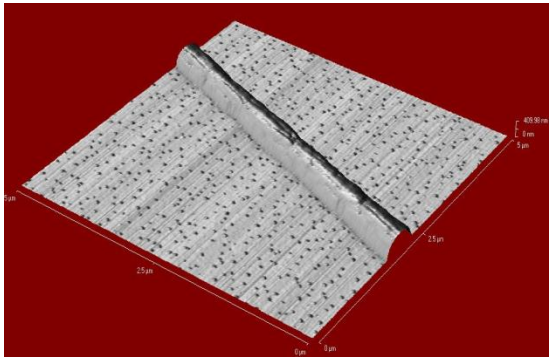

Syringe
Polymer solution
High voltage power supply
Grounded rotating drum

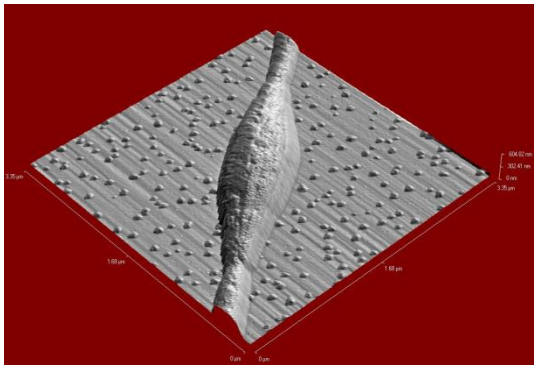
Electrospinning – how to control quality?









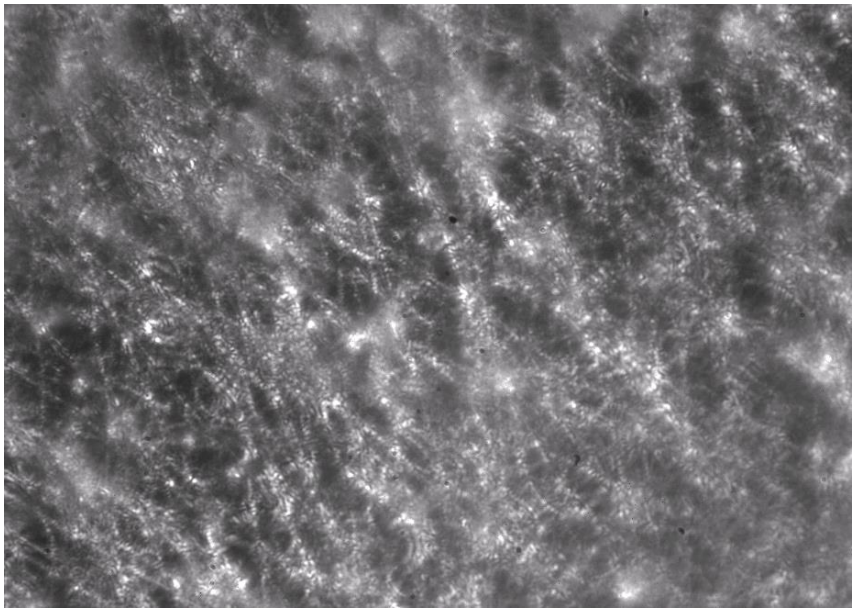


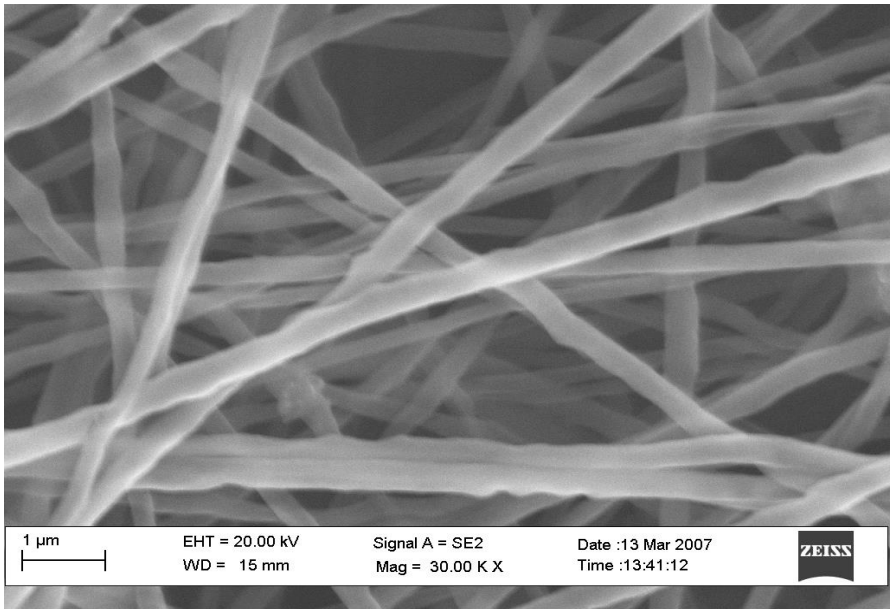
Typical electrospinning of bio-materials.

Polymer	Solvent*	Bioapplications **,***	Comments
DBC (dibutyrylchitine)	ethanol	wound dressing	biocompatible, helps heeling process
TAC (triacetylcellulose)	dichloro-methane	wound dressing	
PEO (poly ethylene oxide)	water/ethanol	artery embolization	needs cross-linking, biocompatible
PCL (poly caprolactone)	chloroform	bioresorbable polymer scaffolds for artificial tissues or organs	Biodegradable, biocompatible
P-3HB (poly 3-hydroxybutyrate)	2,2,2-trifluoroethanol	bioresorbable polymer scaffolds, artificial tissues or organs	biosynthesized, biodegradable, biocompatible
Protein (BSA)	water	FRET sensor, wound dressing	
Fluorescent labeled protein	water	disease diagnostic on a cellular level (by FRET technique)	

Method opens atractive possibility to compose nanostructure of diverse bio materials

Electrospinning of bio-materials.

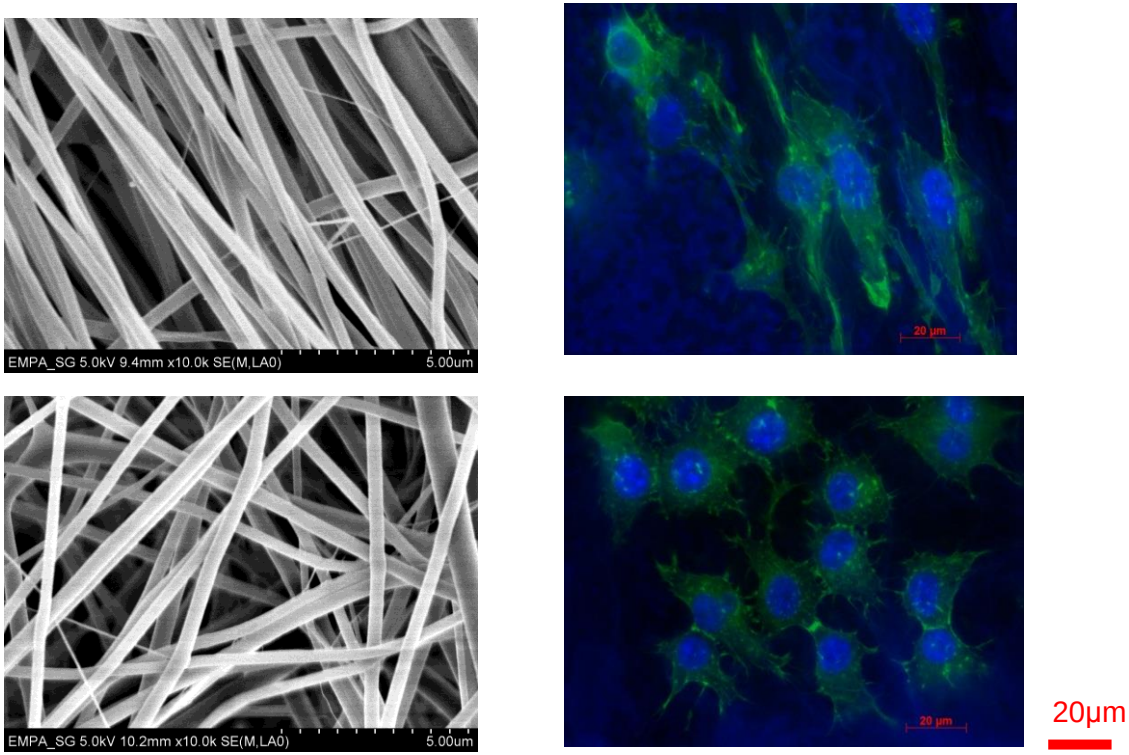




Nanofibre matrix PCL/PEO on aluminium foil and SEM image

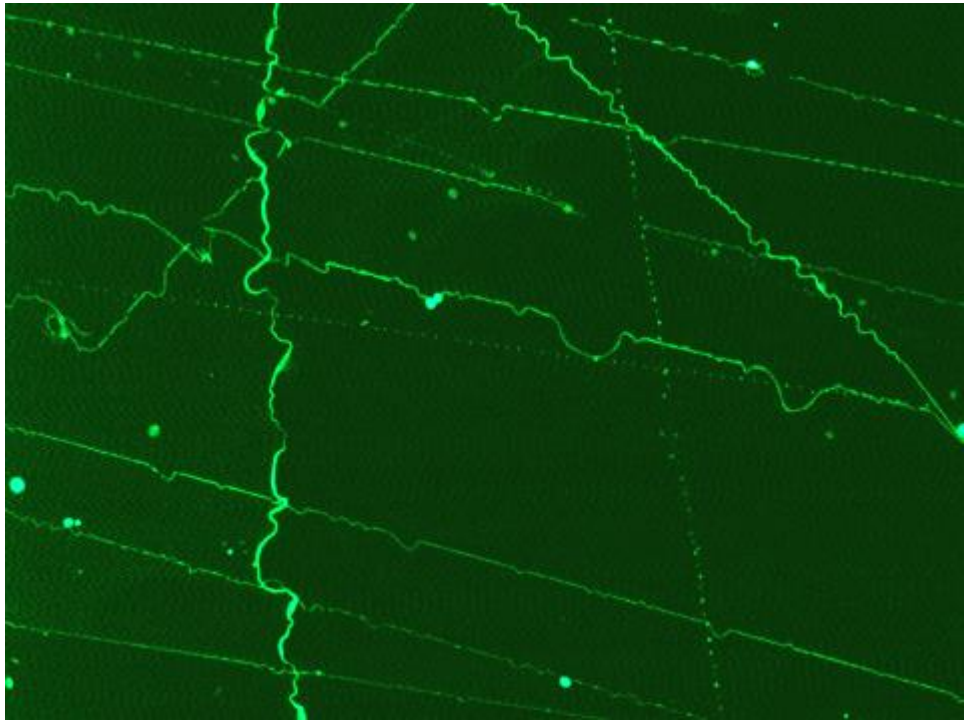
Scaffolds of poly-caprolactone (PCL), poly-3-hydroxybutyrate (PHB), and copolymer poly-3-hydroxybutyrate-co-hydroxyvalerate (P-3HB). Nanofibres porosity to promote cell adhesion and proliferation. Possible use for modification of Bioglass foams.

Polycaprolactone/gelatin scaffolds



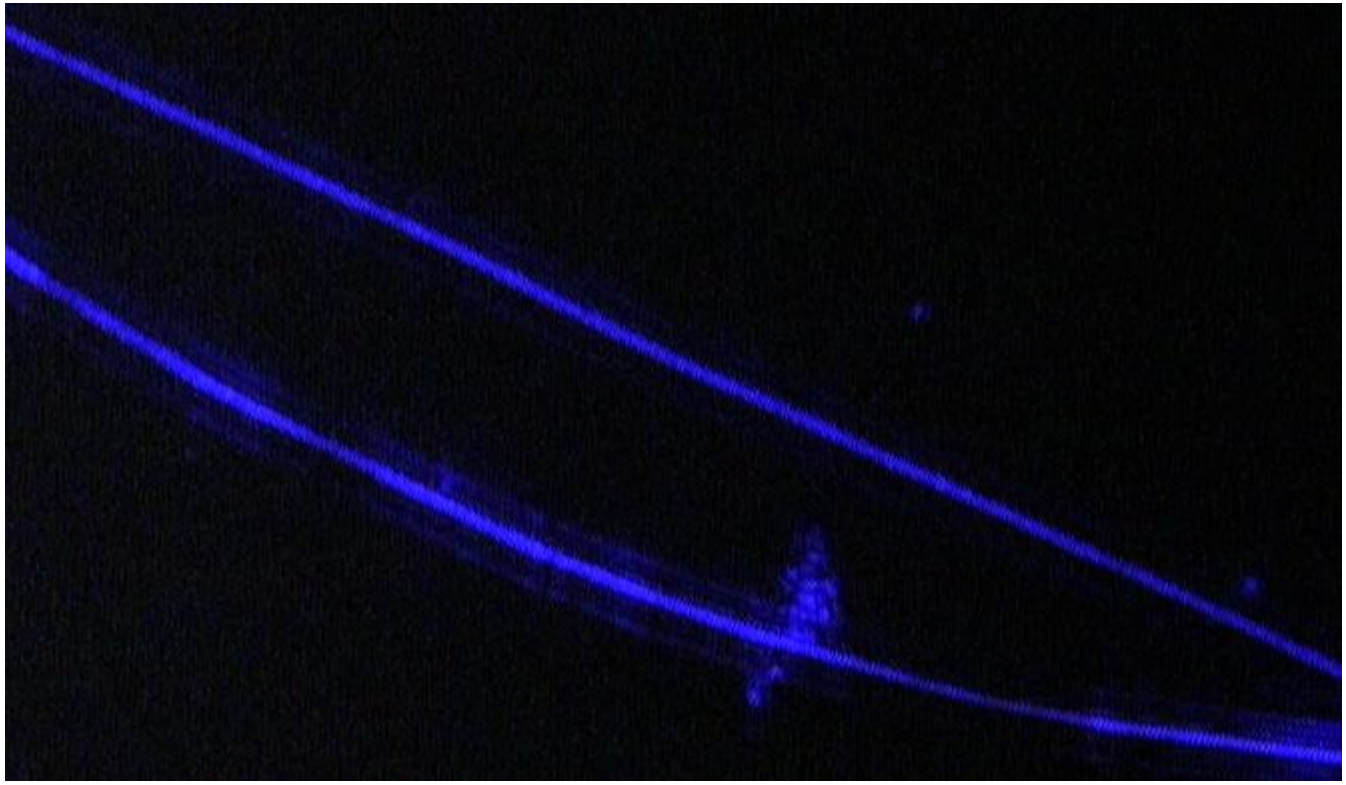
SEM and fluorescent microscope images of 3T3 fibroblasts on PCL/gelatin scaffolds after 4 days in vitro conditions.
Effect of fibers orientation

Electrospinning of bio-materials.
Fluorescence tagging



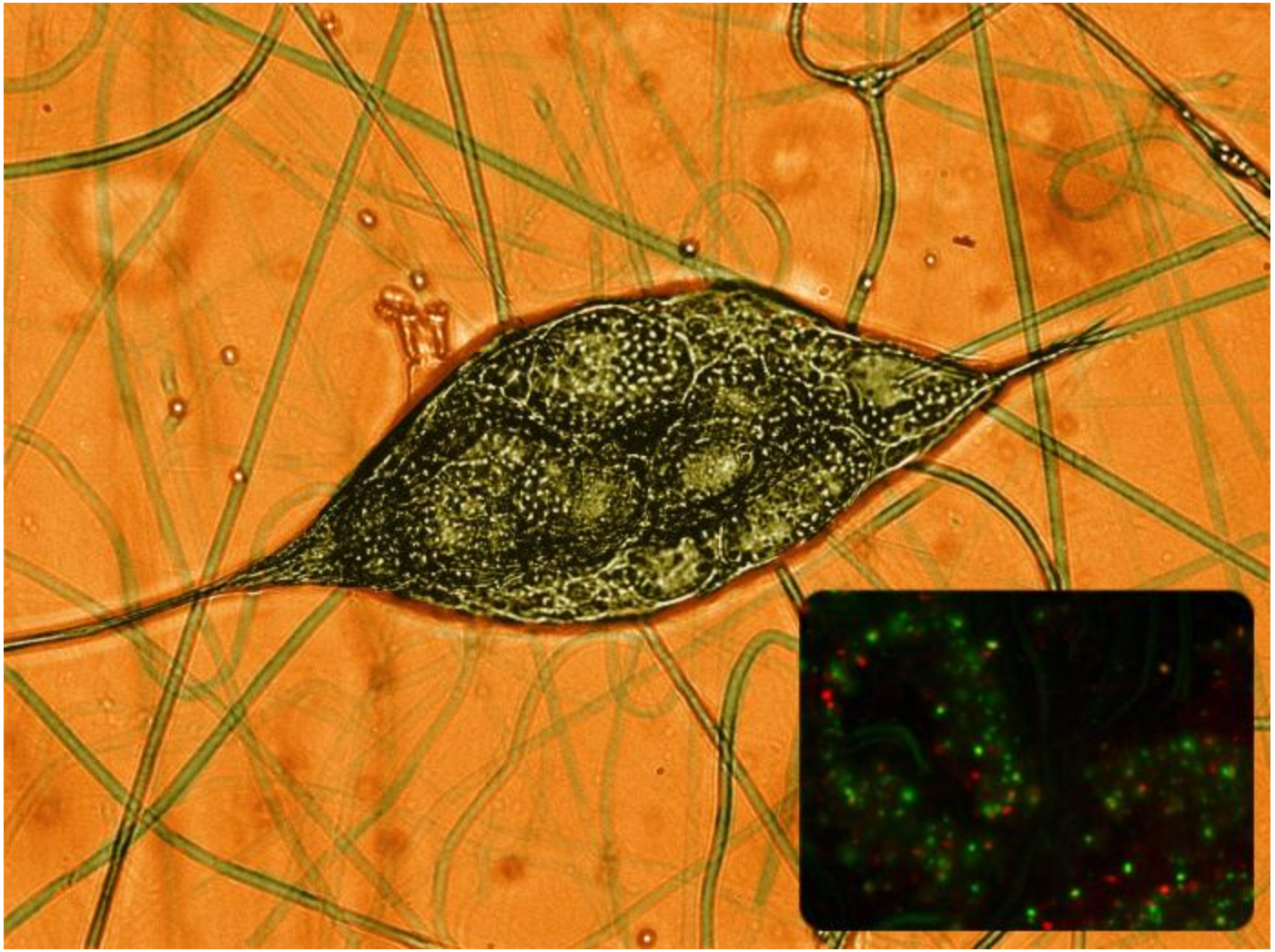
Nanofibre made of Bovine Serum Albumin (85%) and poly(ethylene oxide) (15%), water solution. Labelled with fluoresceine isothiocyanate (FITC).
Demonstrated as pH sensor

Electrospinning for quantum - wires.



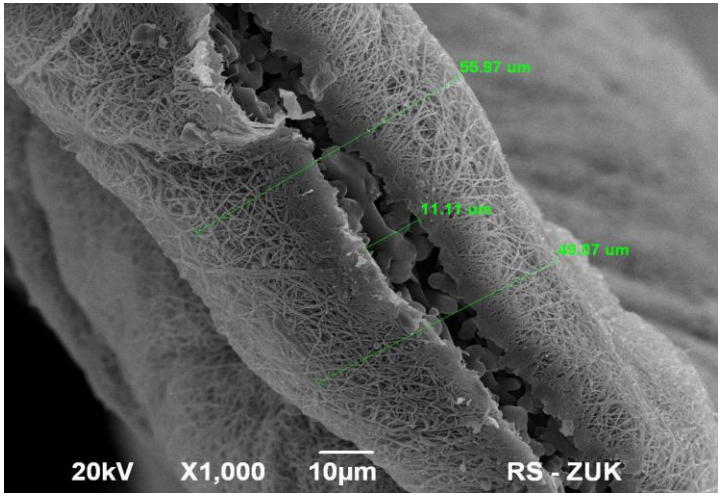
Fluorescence microscopy image of ZnO quantum wires organized in an electrospun fibre. Temperature and pH sensor

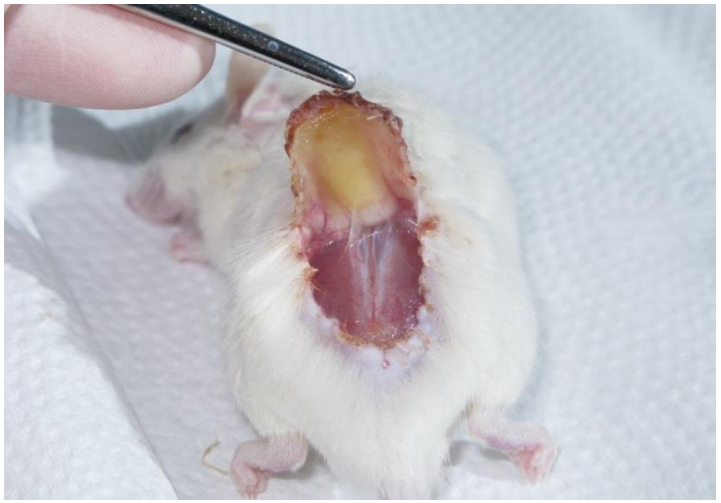
Electrospinning living cells



Encapsulated yeast cells

Electrospun membranes used as wound dressing material

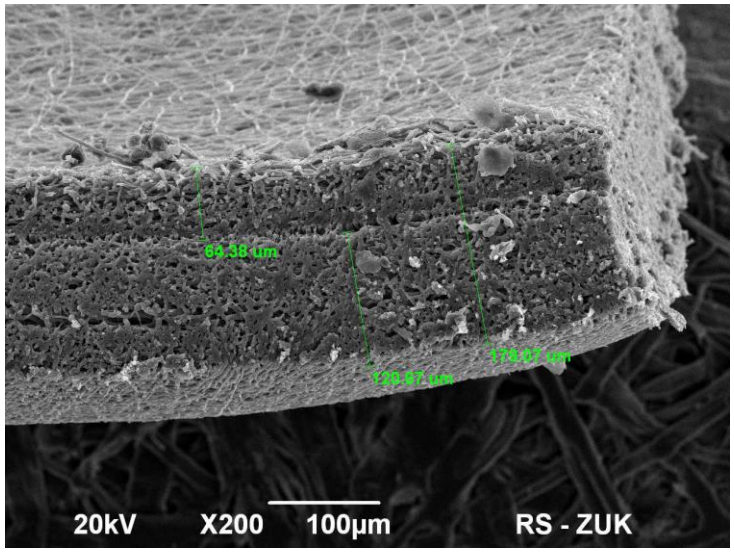




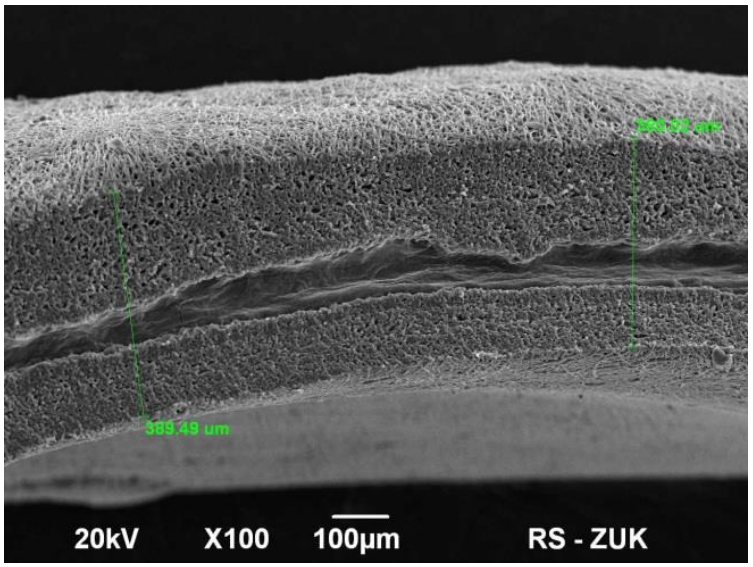
B.Noszczyk

SEM micrograph and application *in vivo* of nanofibrous membrane made of Human Serum Albumin mat

Electrospun membranes used in urology



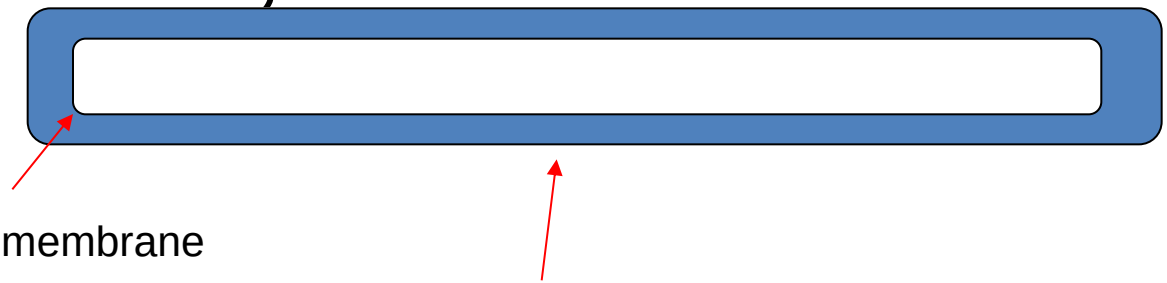
PLCL nanofibrous mat



PLCL nanofibrous mat surrounding amniotic membrane

SEM micrographs of nanofibrous membranes made of PLCL, used for *in vivo* experiments on rats, aimed for urinary bladder wall reconstruction

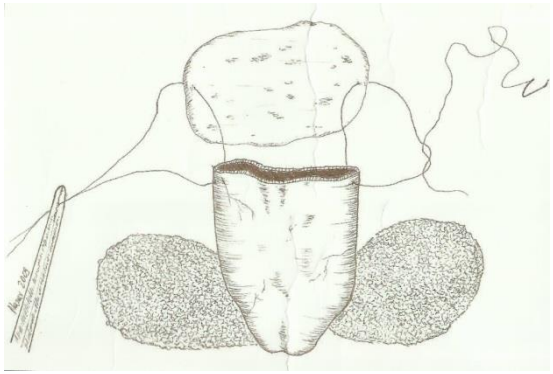
Biocomposite made from amnotic membrane and PLCL, seeded with stem cells



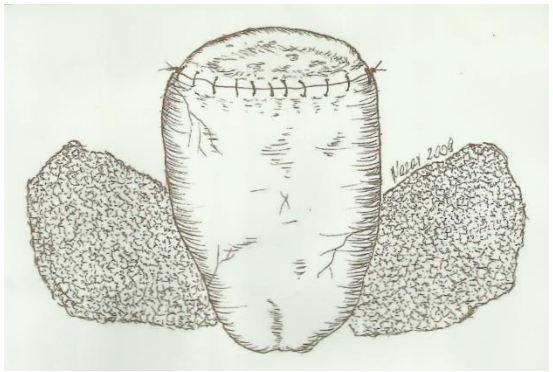
Amnotic membrane

PLCL

10 rats underwent hemi - cystectomy. The gap (0,7 cm^{2}) in the urinary bladder doom was augmented using *in vitro* constructed graft.



J. Adamowicz

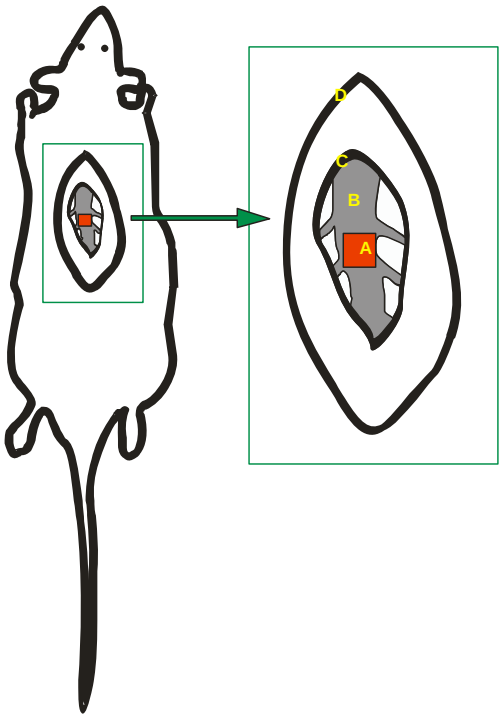


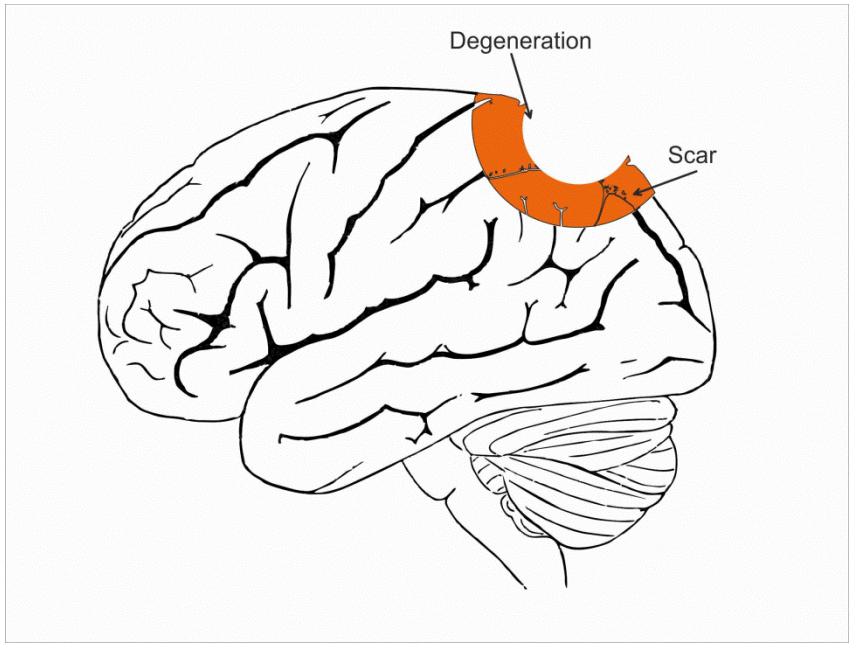
J. Adamowicz

Applied multi layer graft composition is supposed regenerate urinary bladder wall that stand requirement for normal bladder tension development, contraction, elasticity and compliance.

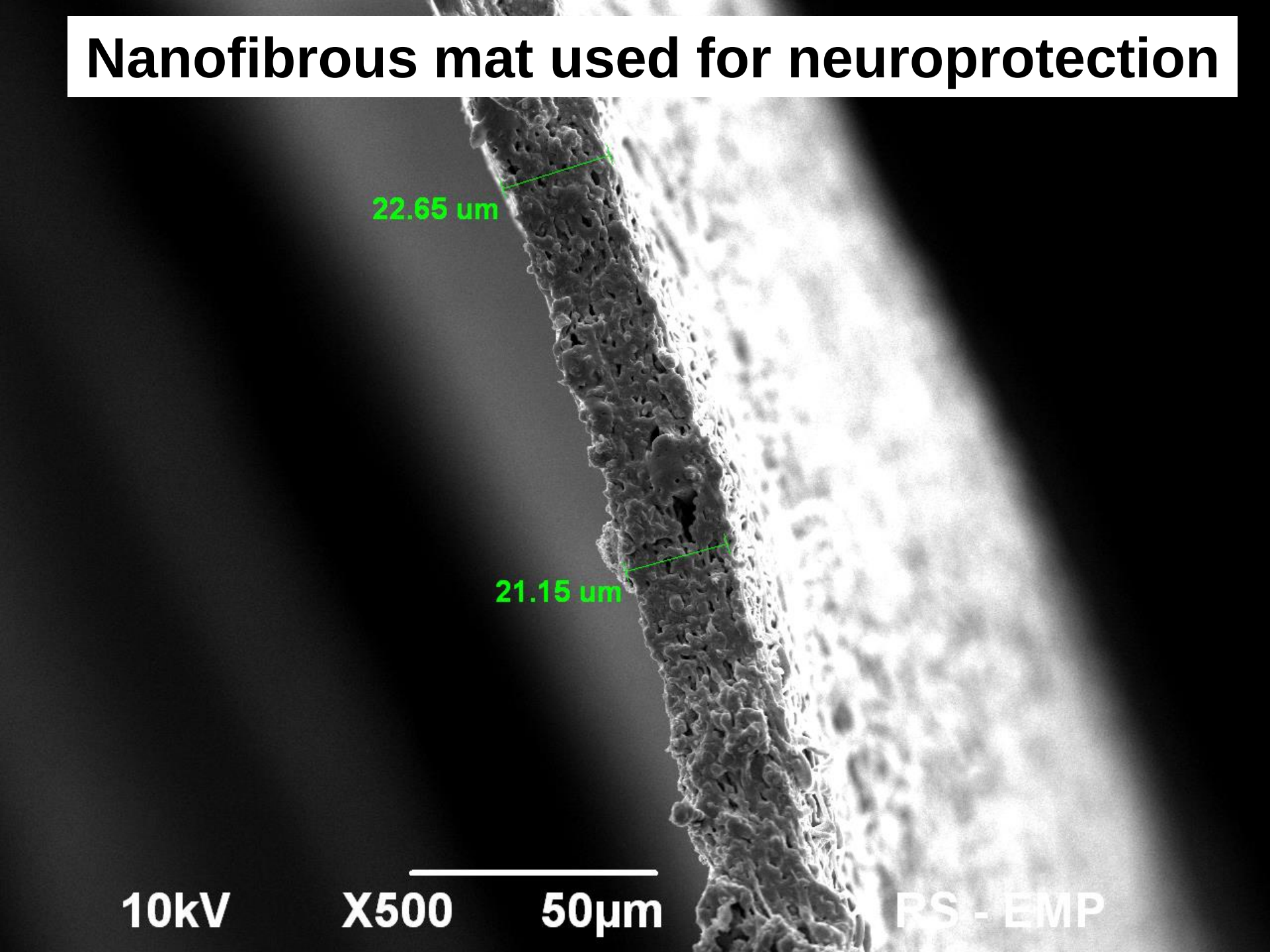
Nanofibrous mats for neuroprotection

The lack of effective neuroprotective products for postoperative treatment of spinal cord or brain injures that lead to scar tissue formation and in worst case to death of the patient.

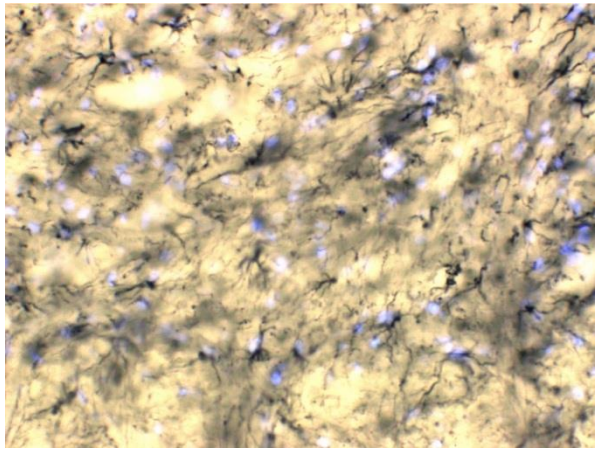




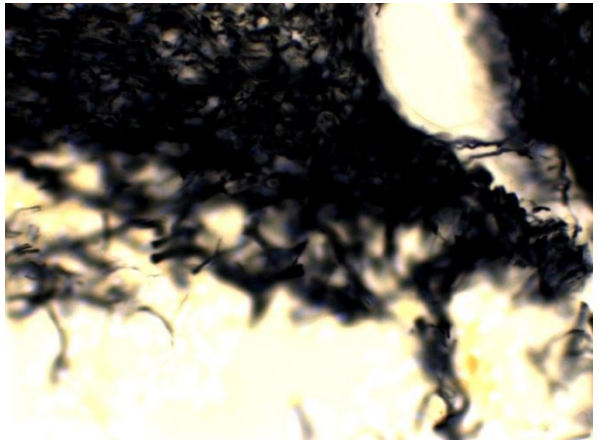
Nanofibrous mat used for neuroprotection



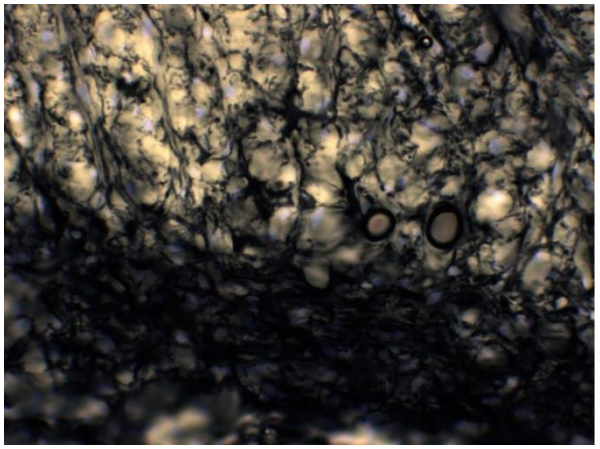
Electrospun mat used for prevention of an excessive cicatrization after neurosurgery – animal model



Intact spinal cord



Injured untreated spinal cord

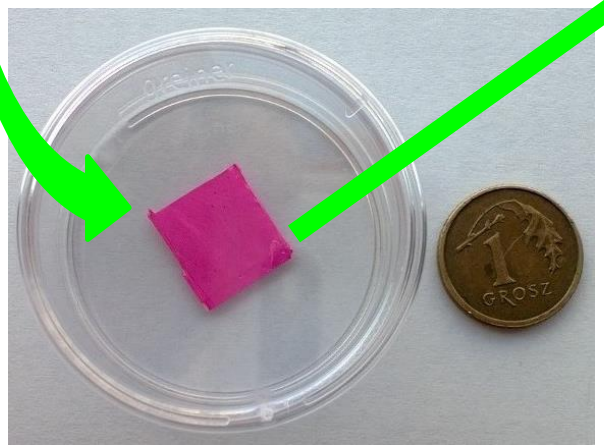


Injured treated spinal cord

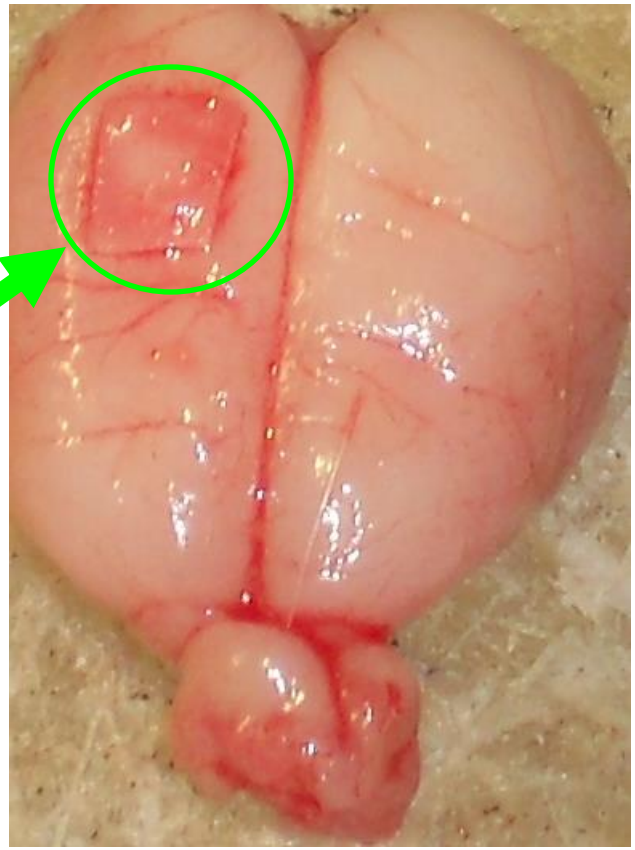
Ultrastructural features of spinal cord protected by neuoprotective electrospun wound dressing. Glial fibrillary acidic protein (GFAP) stain. Massive neurodegeneration and shrinkage of cells is partially avoided by nanomaterial application

Nanofibrous mat for brain injury

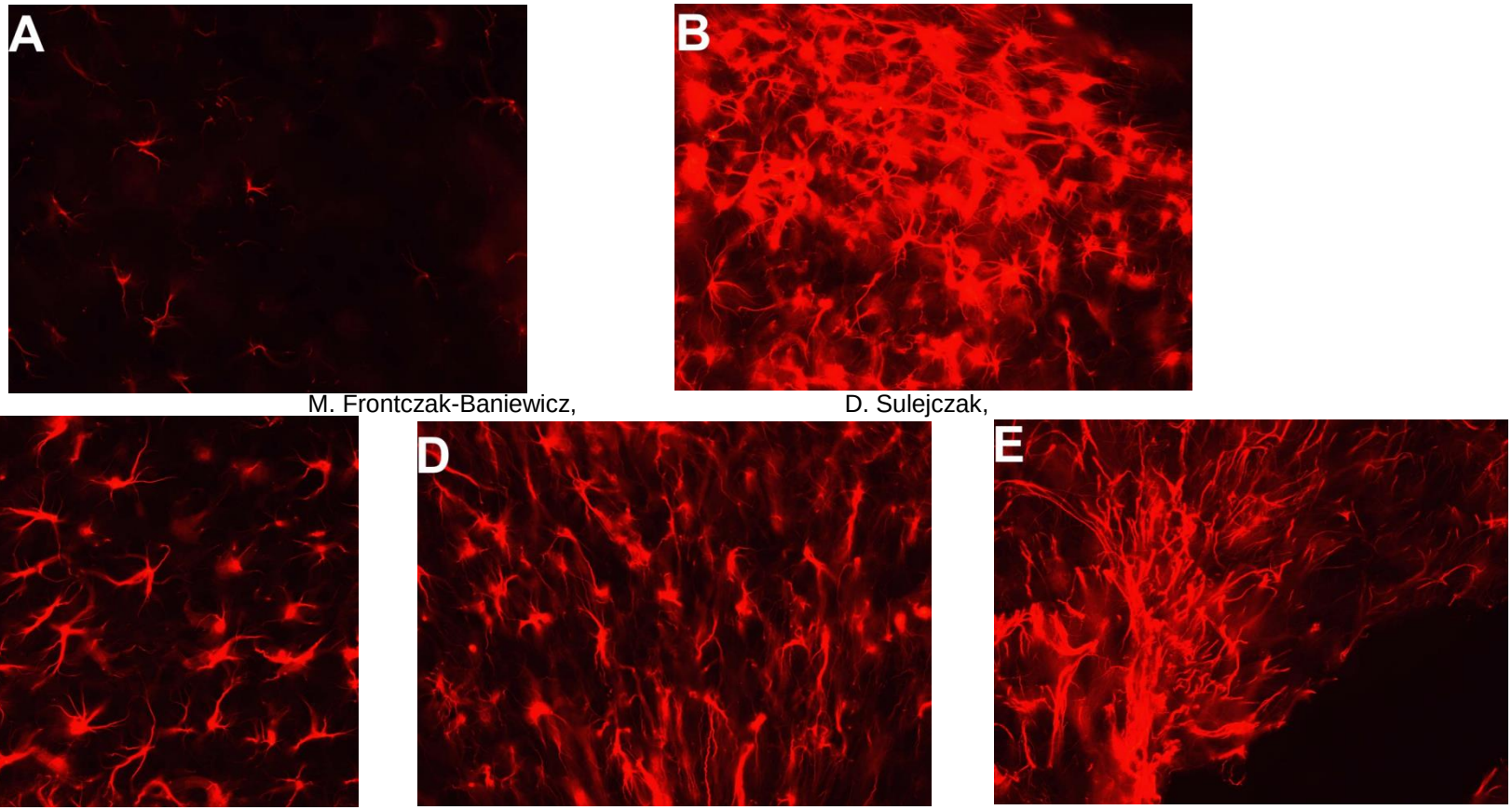
Nanofibrous mat



Dressing on wounded brain tissue



Electrospun wound dressing used for neuroprotection in TBI– animal model

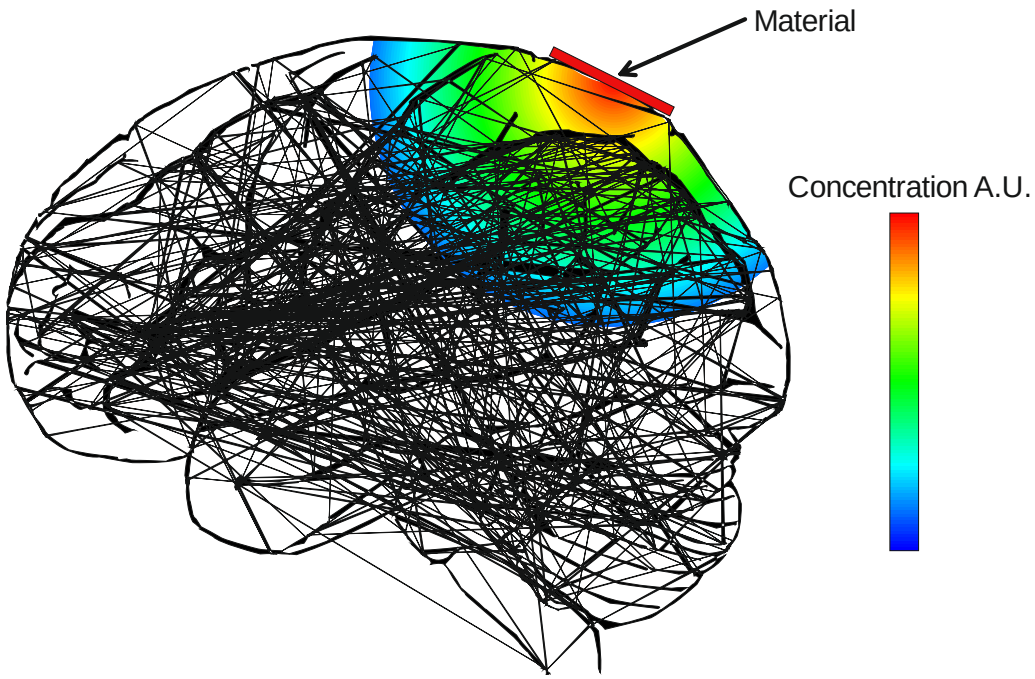


M. Frontczak-Baniewicz, D. Sulejczak,

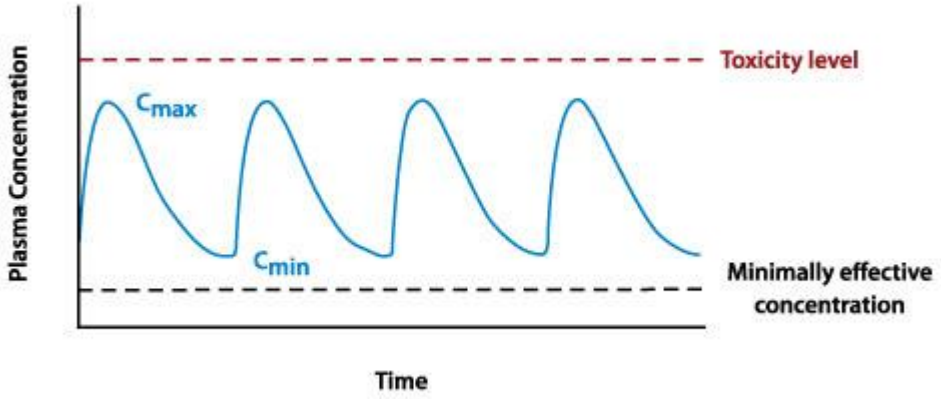
Immunohistochemical features of brain sections from control group(A), unprotected brain injury(B) and protected by neuoprotective electrospun wound dressing(C-4, D-14, and E-30 days post operation. Glial fibrillary acidic protein (GFAP) stain. Hypertophied cells of untreatet glial scar (B), compared to less reactive and more ordered scar treated with nanomaterial application(B-E)

Drugs used in nanofibers

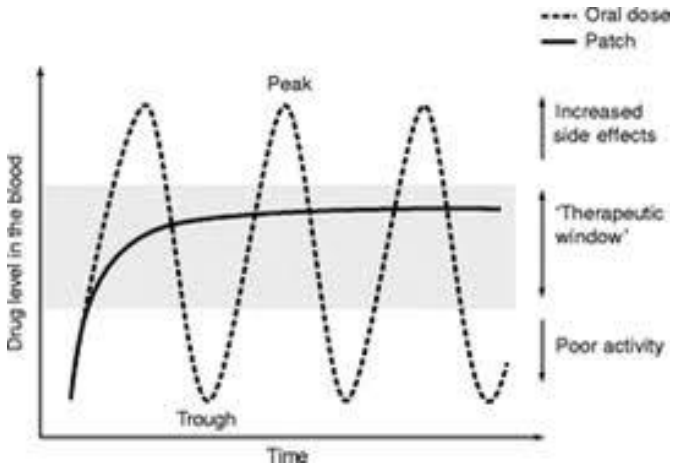
- Vitamin E – antioxidant
- NGF – nerve growth factor
- BDNF – brain derived neurotrophic factor specific for brain tissue



Continuous Drug Delivery System - advantage over multiple dosage



www.saldax.com



www.neurology.org

Concentration profile of a drug administered by multiple injection or oral dosage

Using electrospun nanofibrous mats for continous DDS

Electrospun nanofibers based DDS

Polyesters used for biodegradable polymer matrix

Polyester	Metabolyte
PGA, poly(glycolic acid)	glycolic acid
PLA, poly(lactic acid), polylactide	lactic acid
PHB, poly(3-hydroxybutyric acid)	3-hydroxybutyric acid
PCL, poly(caprolactone)	ω-hydroxyhexanoic acid
copolymers	
PLGA, poly(lactic-*co*-glycolic acid)	lactic acid, glycolic acid
PLCL, poly(L-lactide-*co*-caprolactone)	lactic acid, ω-hydroxyhexanoic acid

Drug Systems

Target	Analog
Lipophilic - solid fiber, core-shell	
α-tocopherol 430Da, r_H = 0,92nm	Rhodamine B 479Da, r_H = 0,9nm
Hydrophilic – core-shell, emulsion electrospinning	
Sodium glutamate 169Da, r_H = 0,56nm	Methylene Blue 320Da, r_H = 0,26nm
Neuron Growth Factor 13,4kDa, r_H = 4,9nm	Bovine Serum Albumin-FITC 66kDa, r_H = 4,65nm
Brain Derived Neurotrophic Factor 13,6kDa, r_H = 2,6nm	
	Gadovist* MRI contrast agent 605Da, r_H = 0,8nm

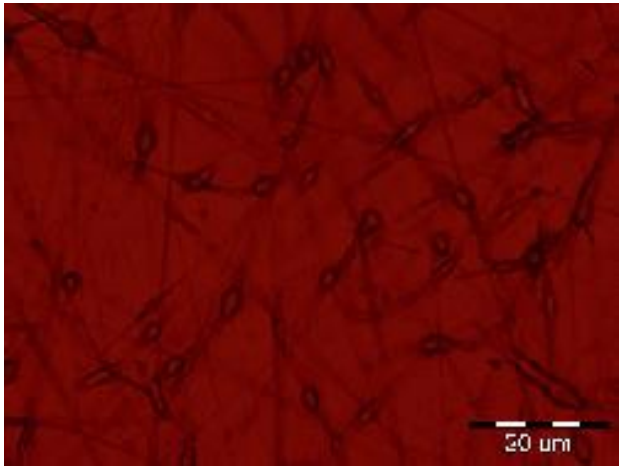
External Fluid Systems

Target	Analog
Cerebrospinal Fluid Volume exchange ≈ 3 times/day	PBS solution At sink conditions (infinite medium)
Brain tissue $D_{tracer} = 1{,}3 \cdot 10^{-11}\ m^2/s$ $k_{elim} = 0{,}014\ 1/s$	PVA – Borax hydrogel $D_{rodB} = 6{,}3 \cdot 10^{-11}\ m^2/s$

How to obtain optimal release profile?

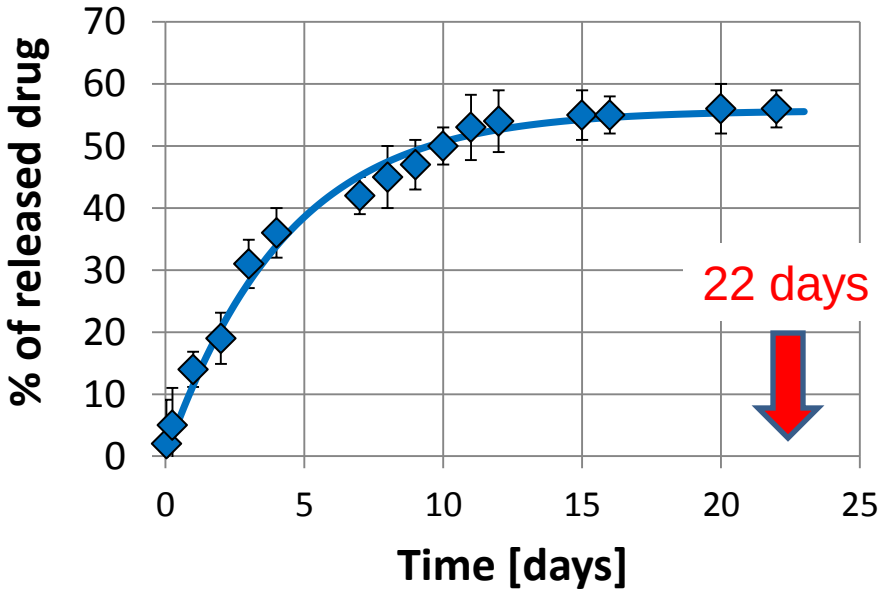
- Selecting desired drug-polymer configuration
- Selecting optimal material structure (porosity, multilayer)
- Verifying release profiles for „analog system” and targeted one
- Modeling, verifying and validating models

Analog system



Fluorescence microscopy of encapsulated Rhodamine B

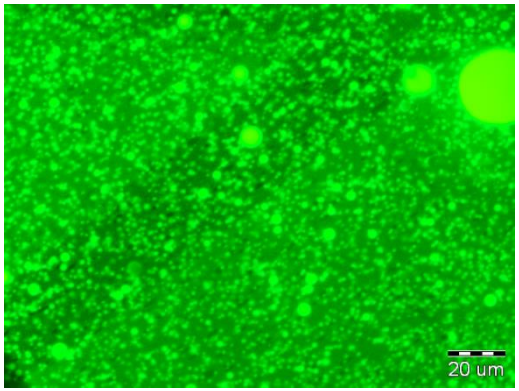
Targeted system



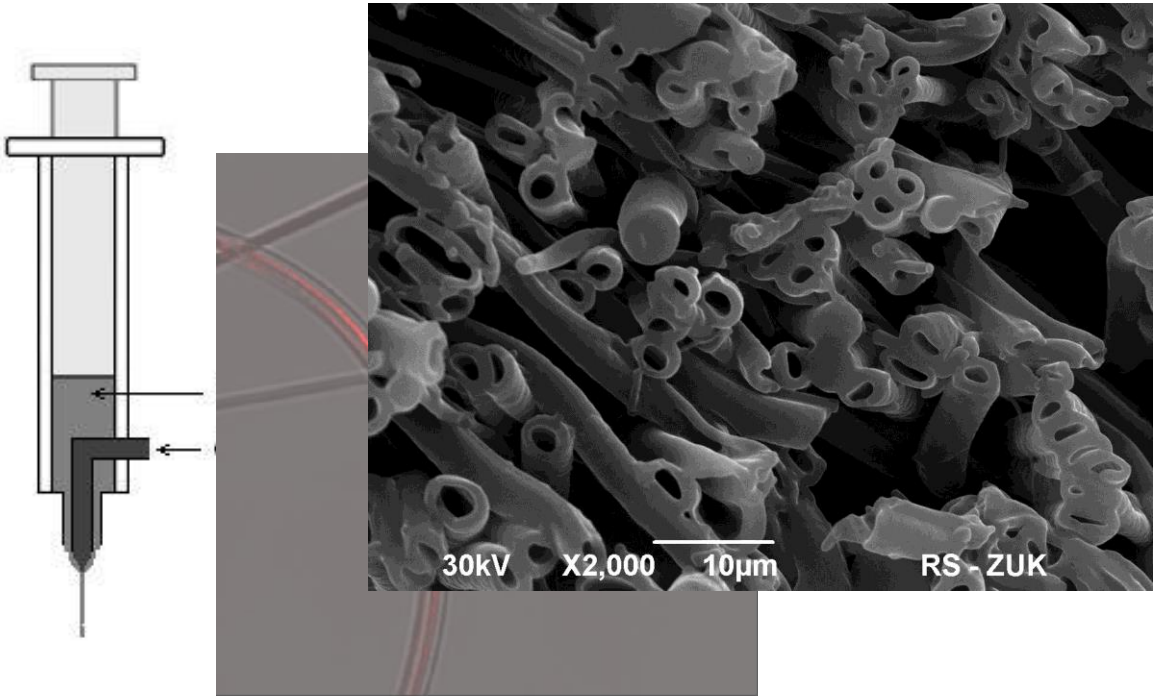
Release profile of α-tocopherol from PLCL fibers

Drug encapsulation methods

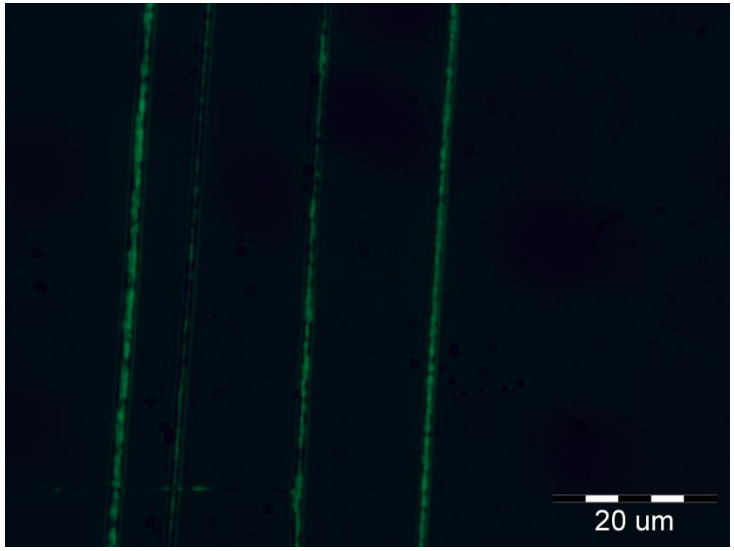
- Solid fibers (lipophilic drugs)
- Emulsions (hydrophilic drugs)
- Core-shell (hydrophilic, lipophilic)



W/O emulsion from fluoresceine in PLCL solution



Rhodamine B loaded core in PLCL shell
Confocal microscopy



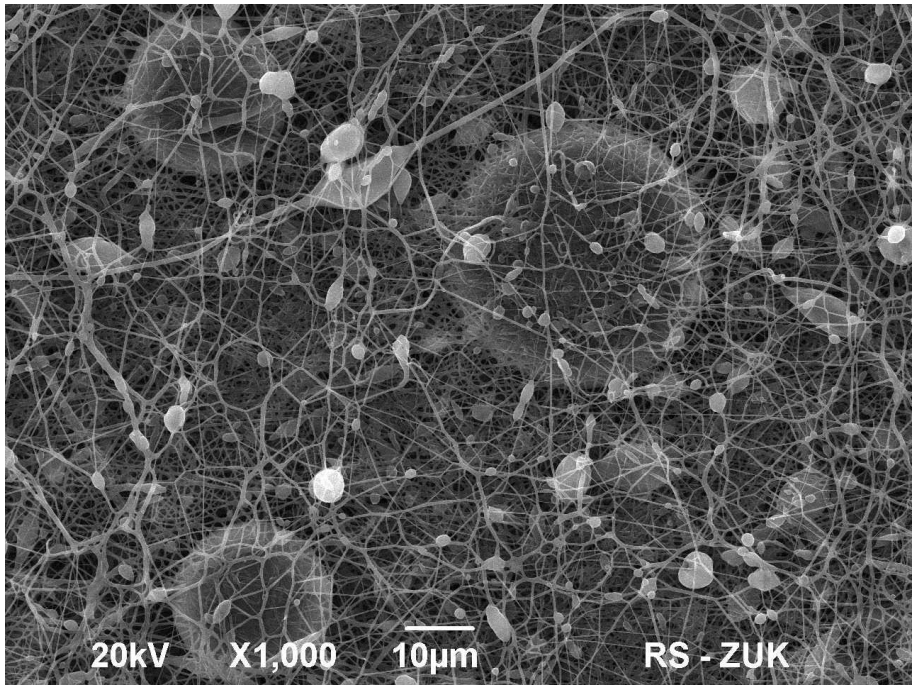
Nanofibers made by emulsion electrospinning

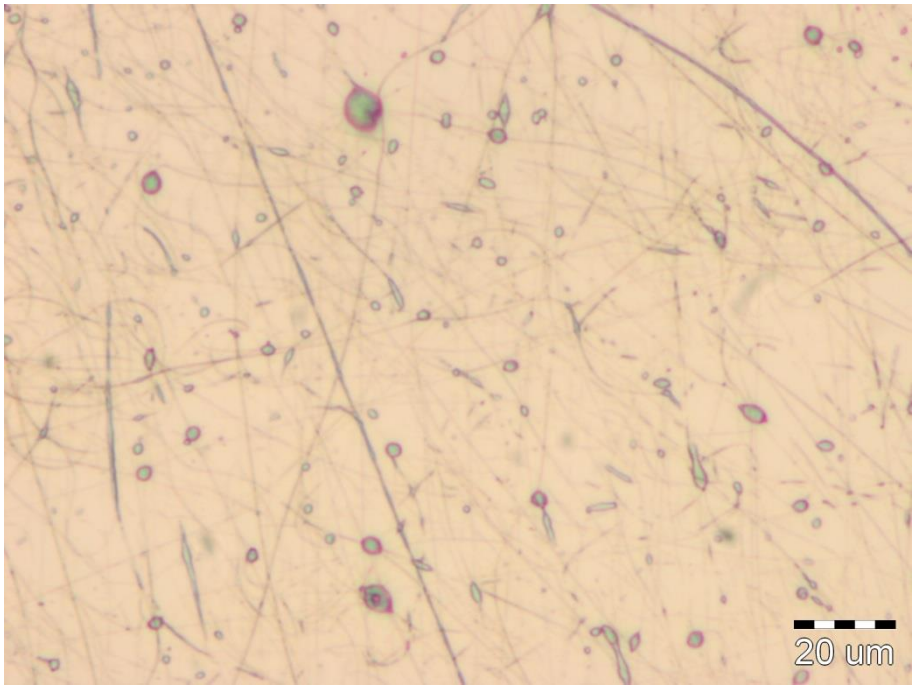
Drug Delivery Systems

Emulsion electrospun membranes

- Applied polymers: PCL, PLCL, PLLA

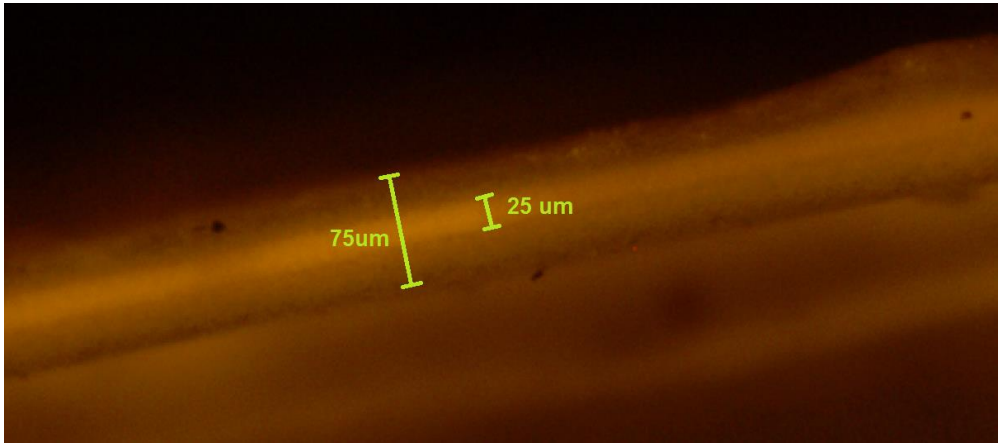
- Fibers with „beads” containing drug or model dye



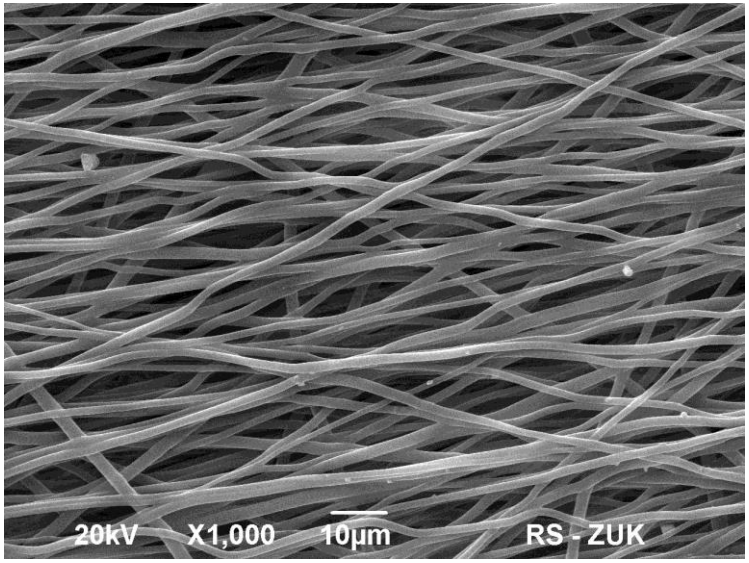


Macroscopic encapsulation

Sandwich membrane multilayer
using aligned nanofibres



Sandwich membrane– middle layer
loaded with Rhodamine B

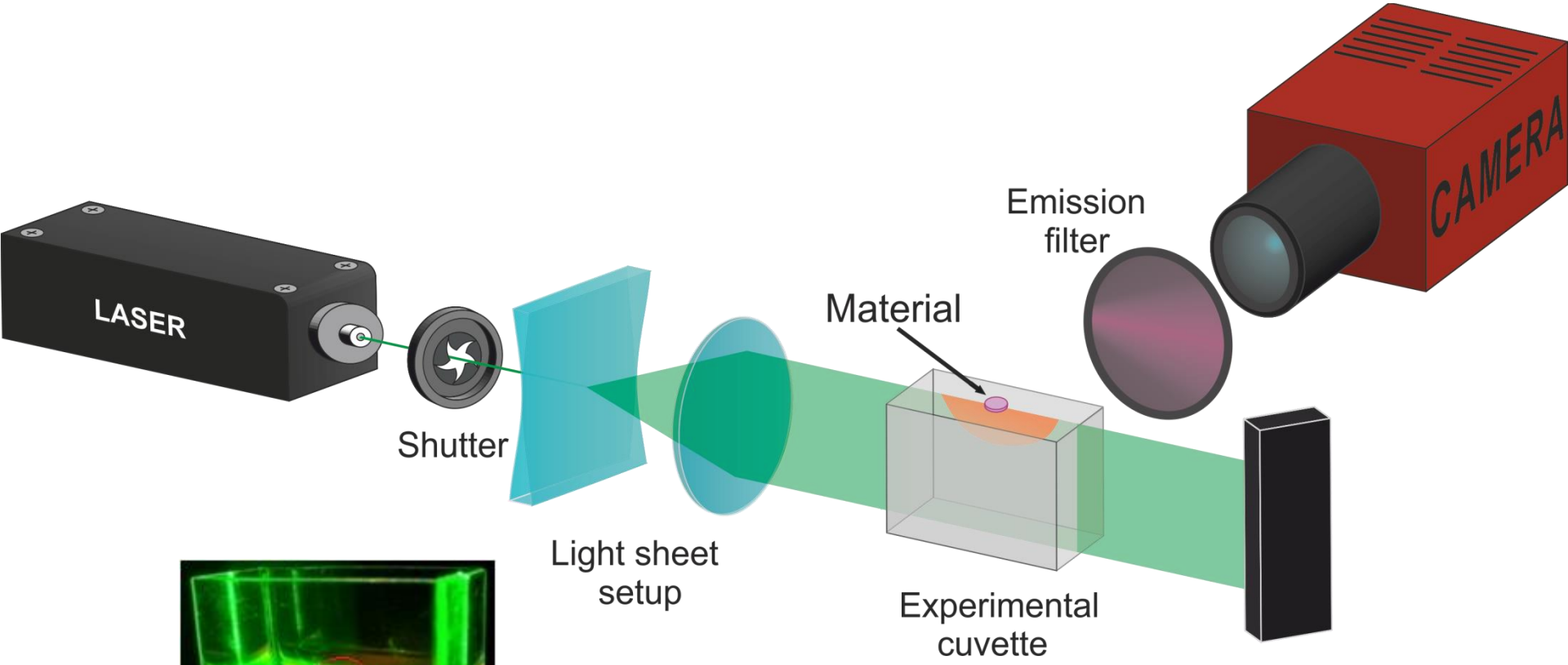


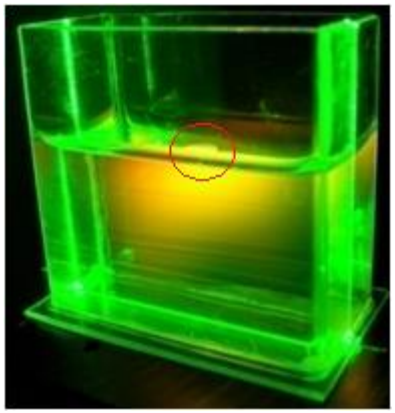
Aligned PLCL nanofibers

Analysing and Modelling

1. Release of the analog drug from nanofibrous mats to hydrogel
2. Diffusion of the analog drug – FRAP optical analysys of the diffusion process in the hydrogel
3. Release of the analog drug from nanofibrous mats to buffer simulating cerebrospinal fluid (spectral fluorymetry)
4. ELISA – enzyme-linked immunosorbent assay – concentration analysis of target drug in buffer simulating cerebrospinal fluid

Experiments with analog drug systems
Rhodamine release from nanofibrous mats





Cuvette with material on the top of the PVA hydrogel

Samples dimension:
Radius = 2,5 mm
Thickness = 150 μm

Experiments with drug analog

quantitative study and diffusion coefficient determination FRAP

Optical measurement at the experimental setup

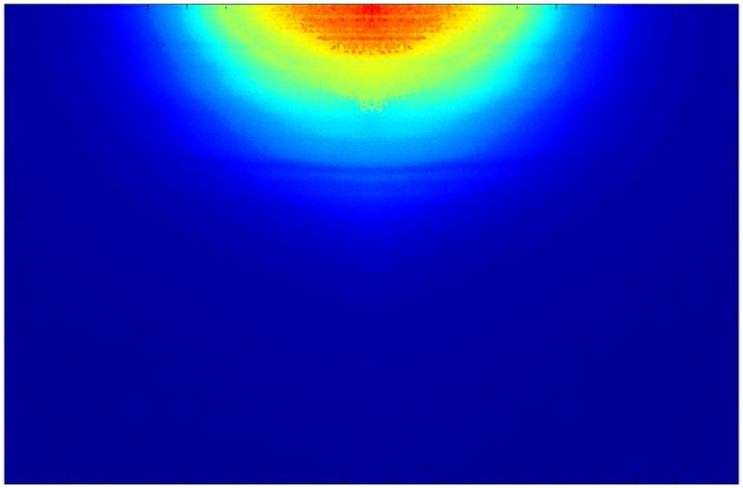
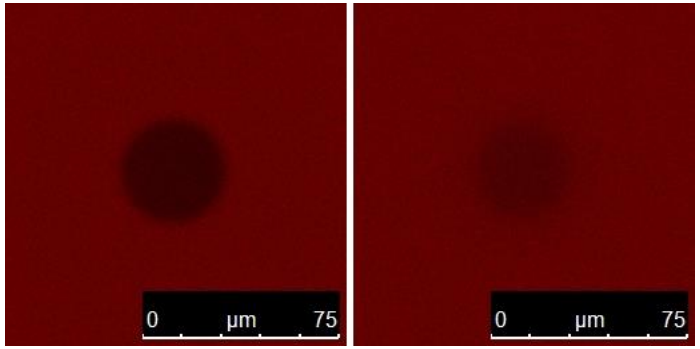


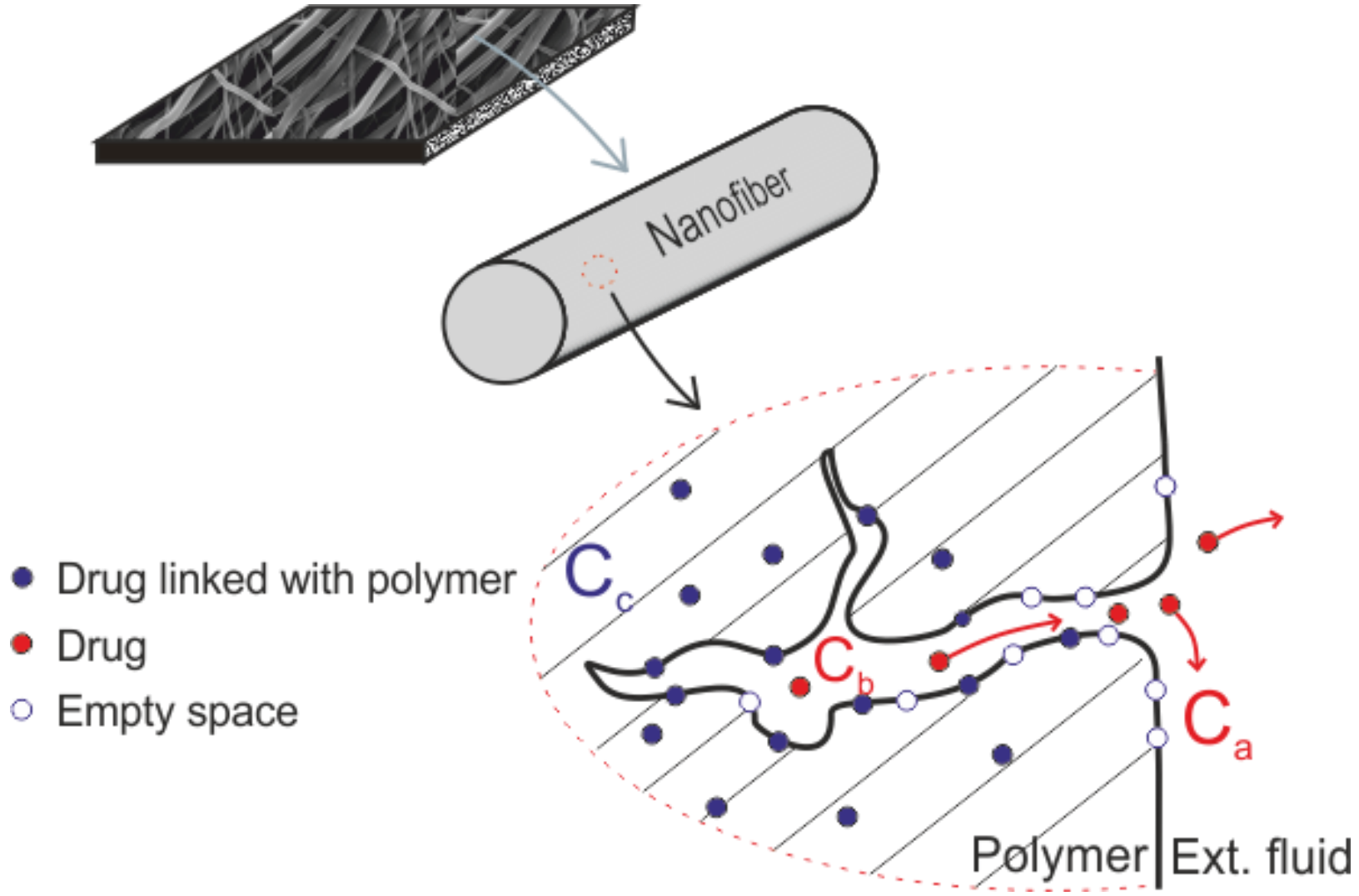
Image from the experiment presenting drug transport from the material

Microscopic measurement FRAP analysis



Diffusion coefficient of Rhodamine B in PVA hydrogel D_{eff} = 6,3 ±1,1 10^{-11} m^2/s

Desorption – diffusion model in porous material



Desorption – diffusion model in porous material

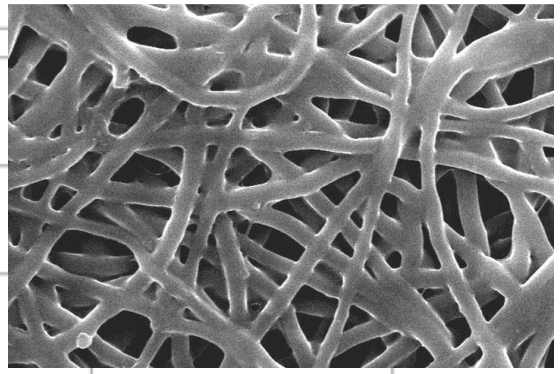
$$\frac{\partial c_A}{\partial t} = k_a \cdot \left(c_A^{\max} - c_A\right) \cdot c_B - k_d \cdot c_A$$

$$\varepsilon \cdot \frac{\partial c_B}{\partial t} = \varepsilon \cdot \nabla \cdot \left(D_B \nabla c_B\right) - \left(1-\varepsilon\right) \cdot \rho_p \cdot \frac{\partial c_A}{\partial t}$$

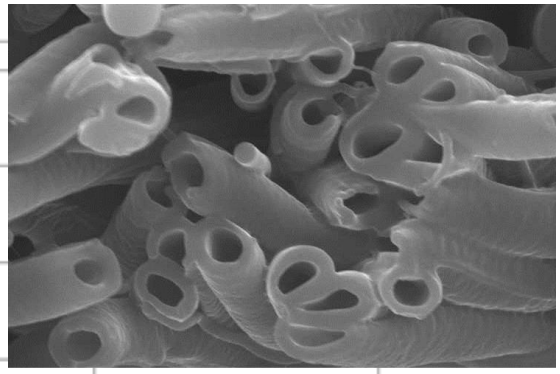
C_A – drug concentration at the nanofiber surface [kg/ kg of the material]
C^{max}_A – maximal drug concentration at the nanofiber surface [kg/ kg materiału]
C_B – drug concentration in the pores of the material[kg/m^3]
ε – porosity of the material[-]
D_B – diffusion coefficient in the fluid [m^2/s]
ρ_p– polymer specific density[kg/m^3]
k_a, k_d – adsorption and desorption constant

Rhodamine B release results in the hydrogel random nanofibers

Diffusion coefficient **D** = 6,3 ·10^{-11} m^2/s
Desorption conts. $\mathbf{k_d}$ = 3,4 ·10^{-4} 1/s
Porosity **ε** = 0,69

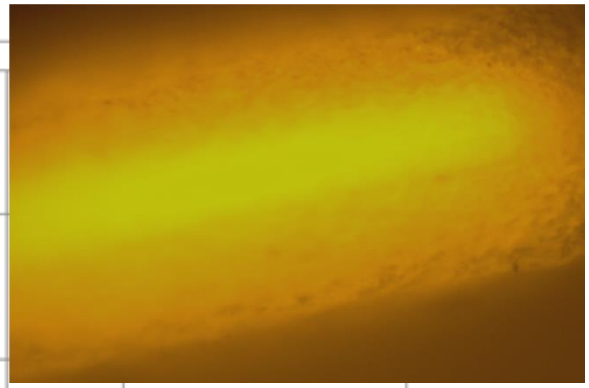


Rhodamine B release results in the hydrogel core-shell nanofibers



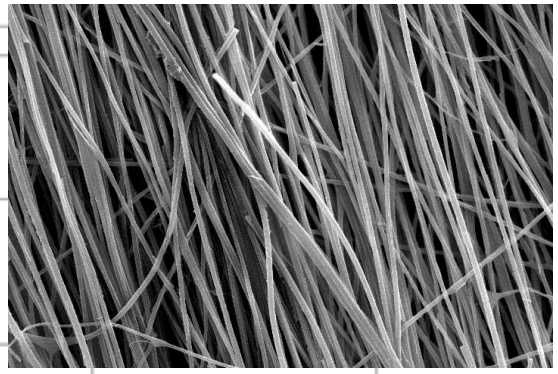
Rhodamine B release results in the hydrogel multilayer

Diffusion coefficient **D** = 6,3 ·10^{-11} m^2/s
Desorption conts. $\mathbf{k_d}$ =4 ·10^{-5} 1/s
Porosity ε = 0,64

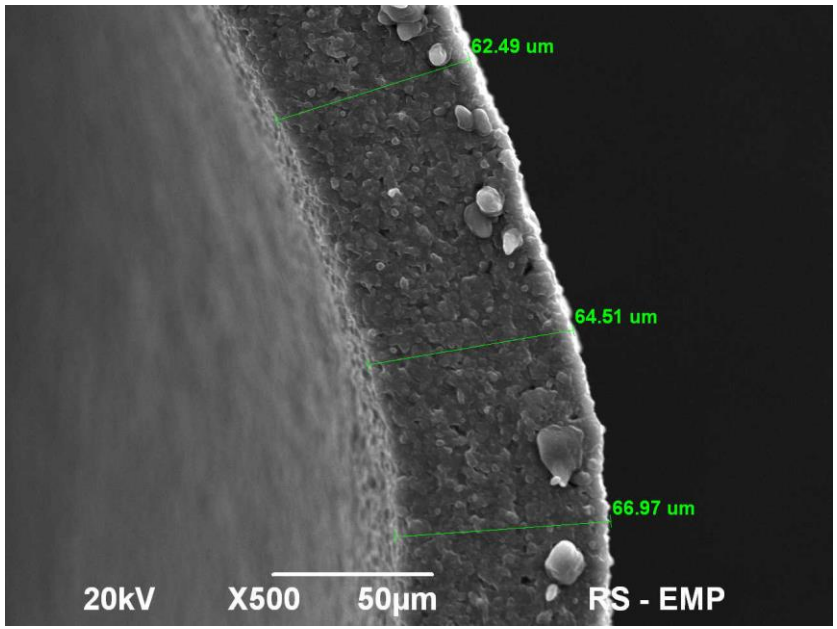


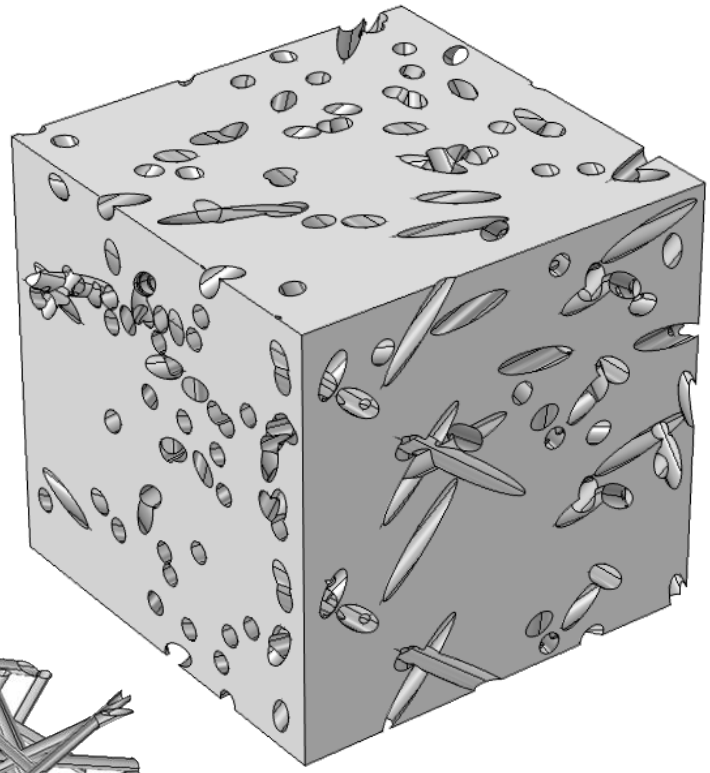
Rhodamine B release results in the hydrogel aligned nanofibers

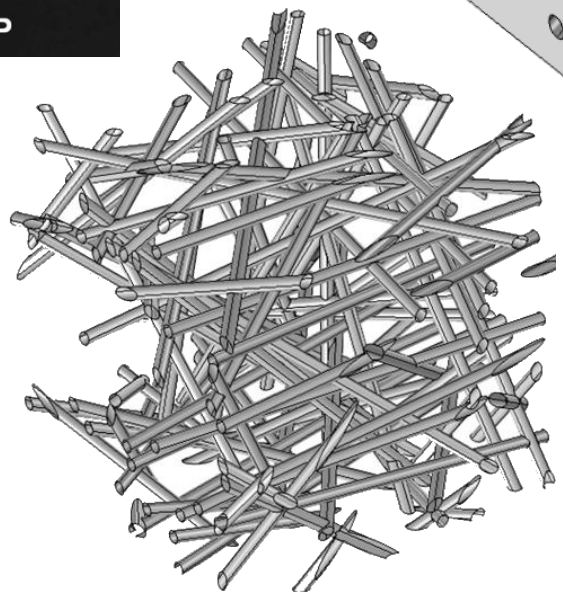
Diffusion coefficient **D** = 6,3 ·10^{-11} m^2/s
Desorption conts. $\mathbf{k_d}$ = 4,1 ·10^{-3} 1/s
Porosity **ε** = 0,12



3D numerical simulations of drug release

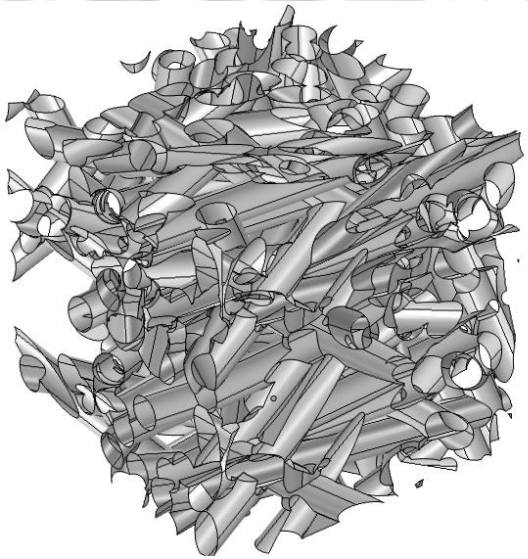


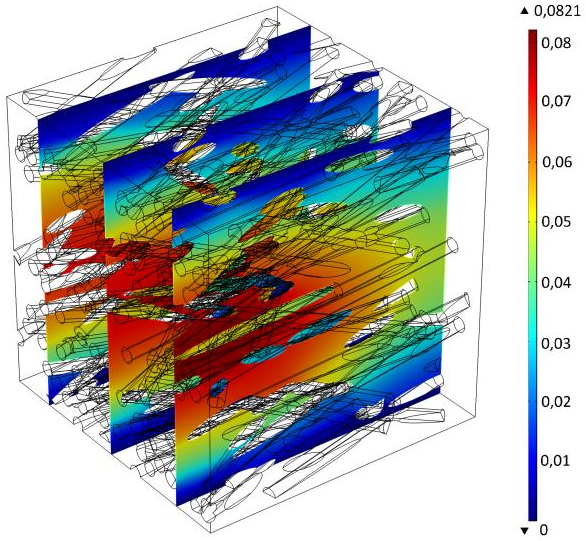




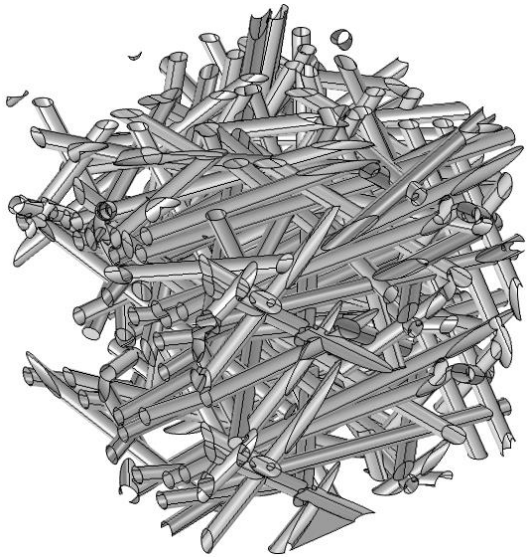
Numerical results for materials with different porosity

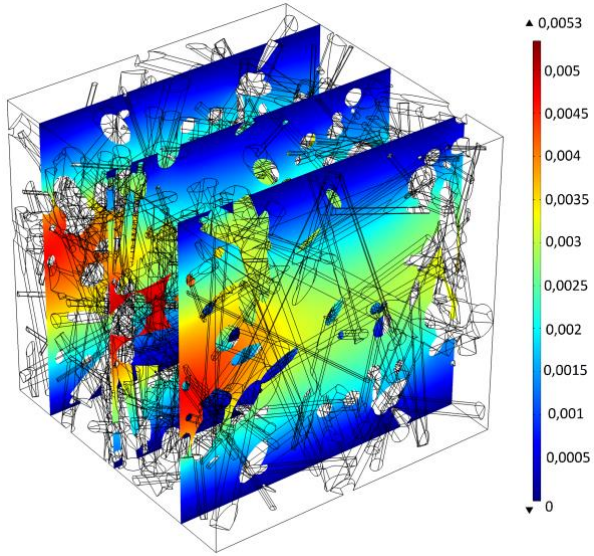
ε = 0,4



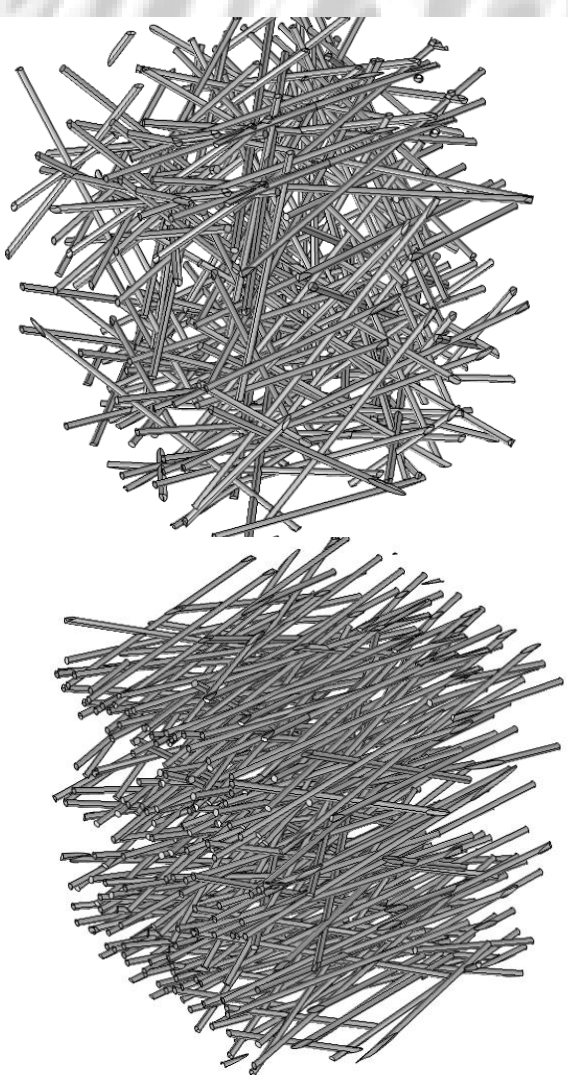


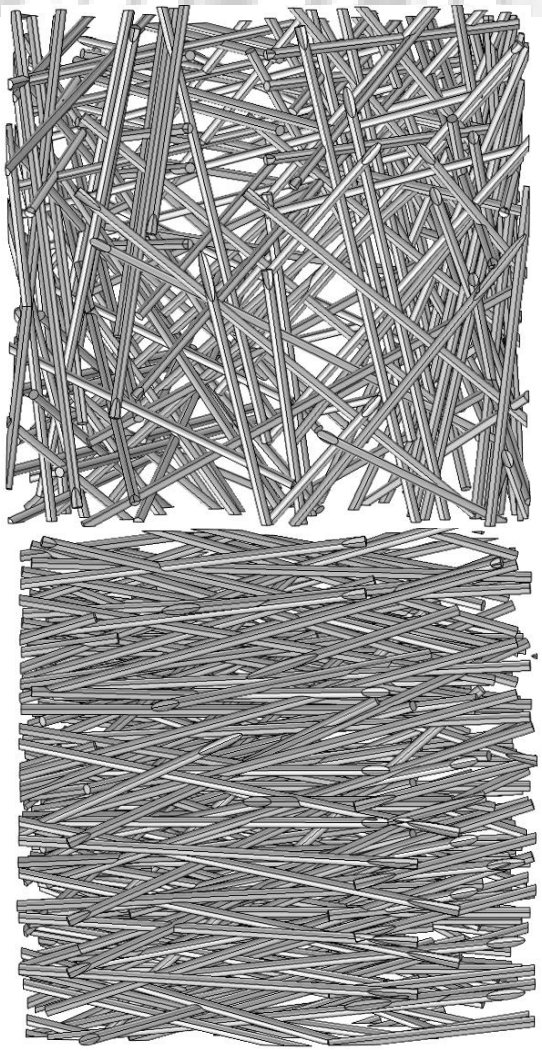
ε = 0,78

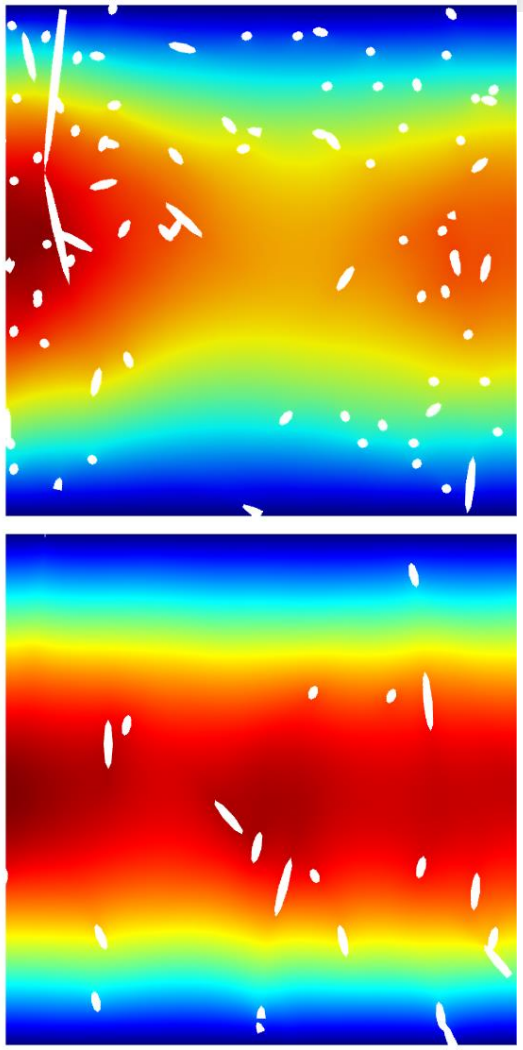




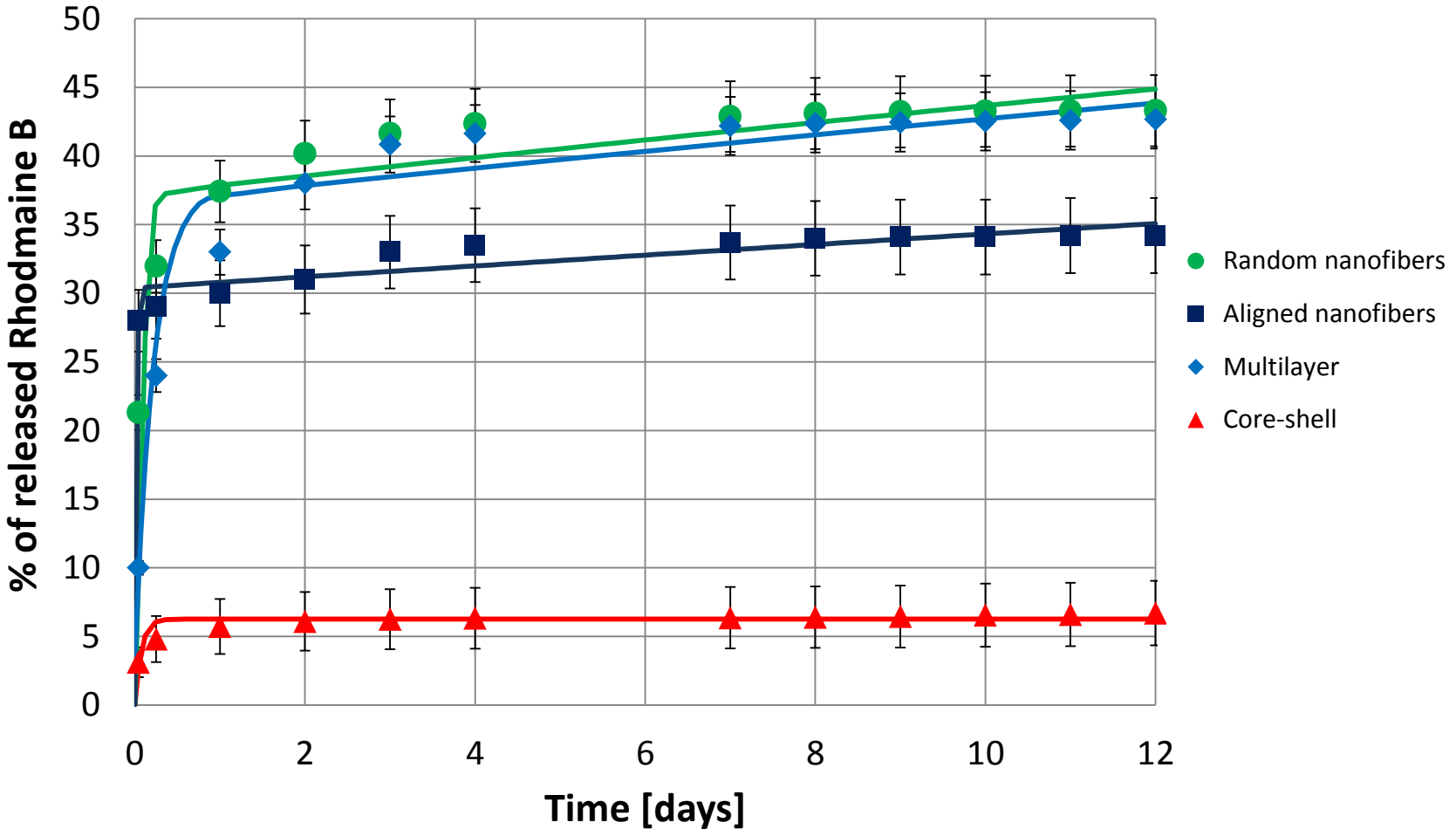
Numerical results for materials with different fibers orientation





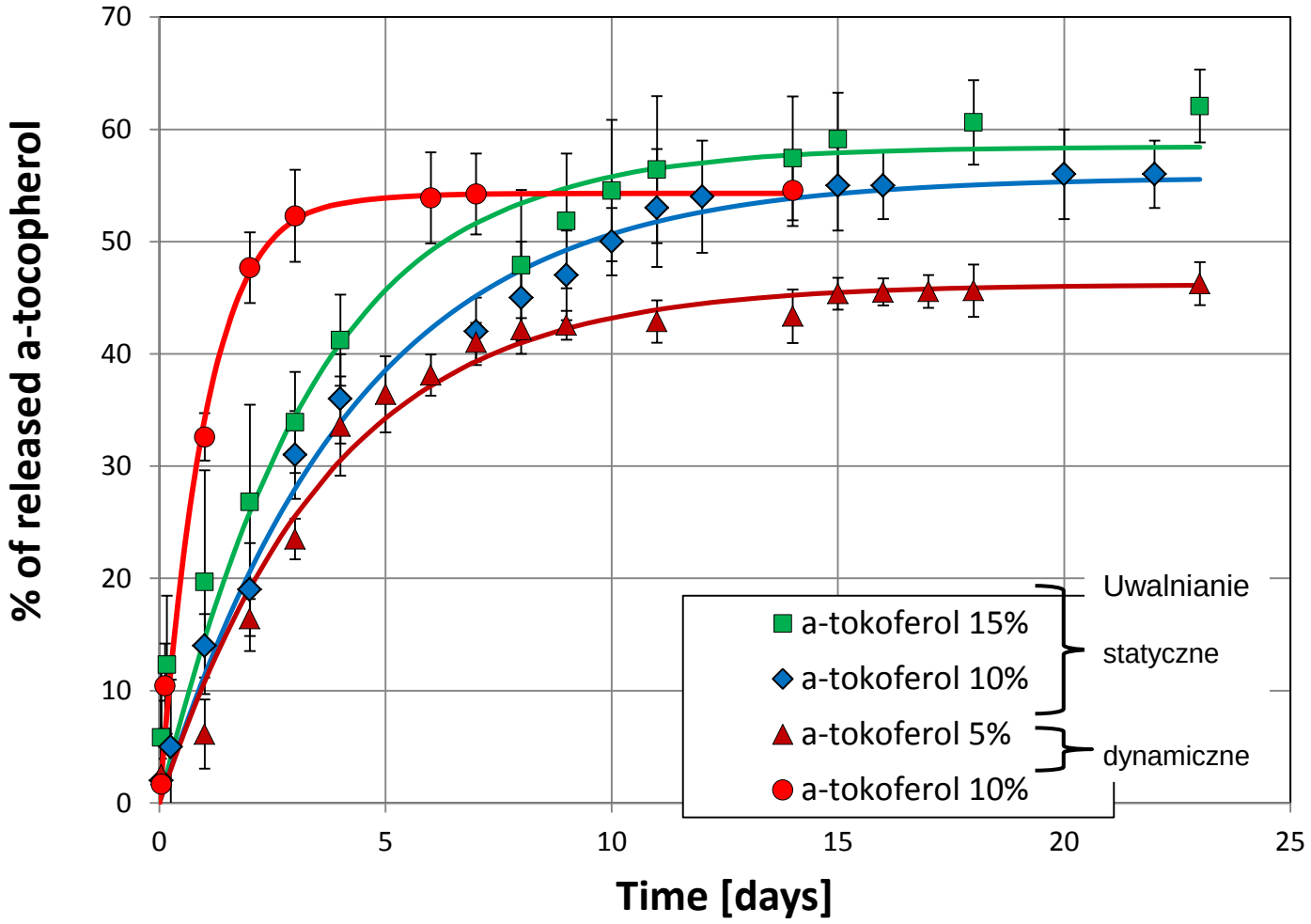


Experimental results of Rhodamine B release in PBS buffer

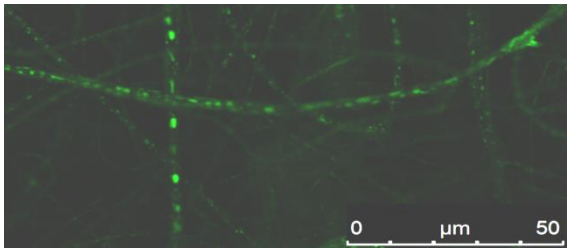


Cumulative release < 100% !

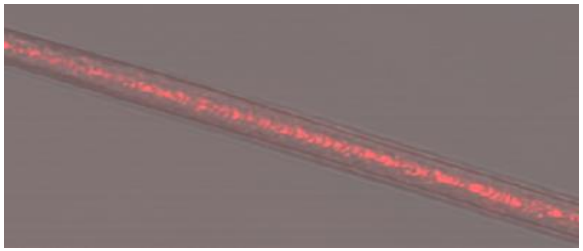
Experimental results of a-tocopherol release



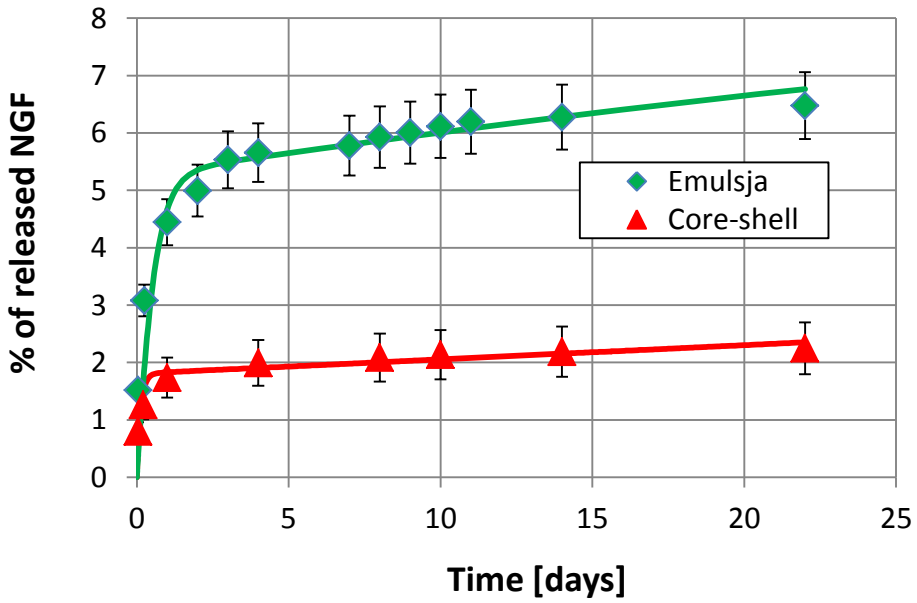
Experimental results of drugs release : NGF and BDNF

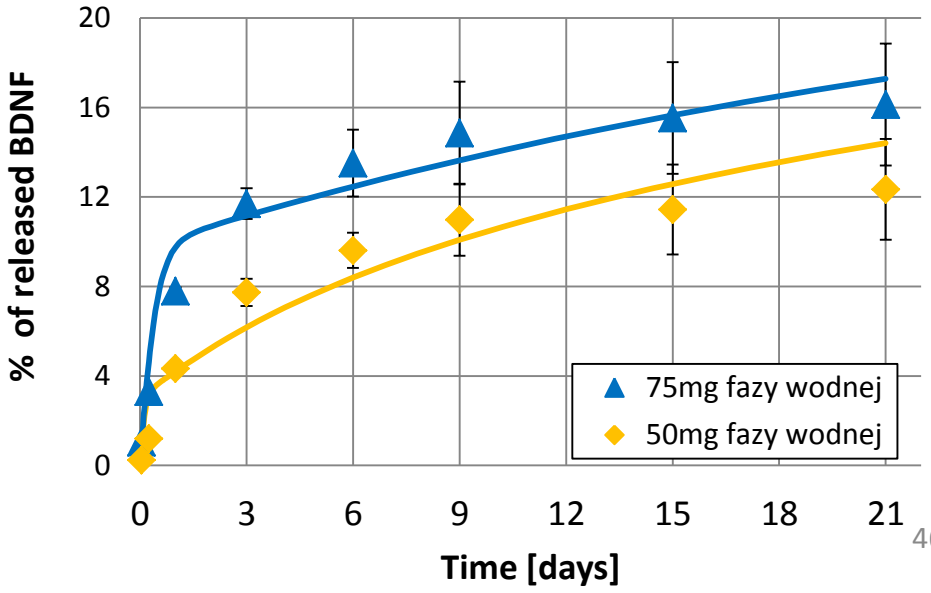


Comparison of NGF release for different type of electrospinning



Comparison of protein release for different amounts of the aqueous phase





Conclusions

- Experimental system is suitable for the evaluation of produced Drug Delivery Systems
- Aligned nanofibers had the bigest burst release and slow release This nanofibers are used to release sodium glutamate to irritate motor neurons to produce rat model for the Amyotrophic Lateral Sclerosis.
- Sandwich membrane can decrease initial burst of drug
- Core-shell nanofibers prolong drug release

	Hydrogel		PBS
Material	**k_{des} [1/s]**	**ε**	**k_{des} [g/cm^2]**
Irregular nanofibers	$3{,}4 \cdot 10^{-4}$	0,69	$1{,}7 \cdot 10^{-9}$
Aligned nanofibers	$4{,}1 \cdot 10^{-3}$	0,12	$5{,}9 \cdot 10^{-9}$
Sandwich mat	$0{,}4 \cdot 10^{-4}$	0,45	$3 \cdot 10^{-10}$
Core-shell	$7{,}5 \cdot 10^{-5}$	0,61	$1{,}5 \cdot 10^{-9}$

Possible commercialization

The potential of the market:

urology, oncology, general surgery and cardiology

Positive results:

- single or multi-drug release in time of weeks
- controlled drug release
- no signs of inmflammation after neurosurgery
- no signs of cell death
- material prevents scar formation

Patents: P.390140 (pending), P.395894 (pending), P.404667 (pending)

Acknowledgments.

- P. Sajkiewicz, T. Chmielewski, A. Molenda, IPPT PAN
-J. Rafałowska, IMDiK PAN

- T. Drewa, T. Kloskowski, J. Adamowicz, UMK Collegium Medicum
- T. Ciach, WUT, IChiP
- R. Stachowiak, WU, Biology Dept.
- B. Noszczyk, MCP

This research is supported by Ministry of Science and Higher Education, NCBiR grant no. R13008110. Paweł Nakielski has been supported with a scholarship from the European Social Fund, Human Capital Operational Program.