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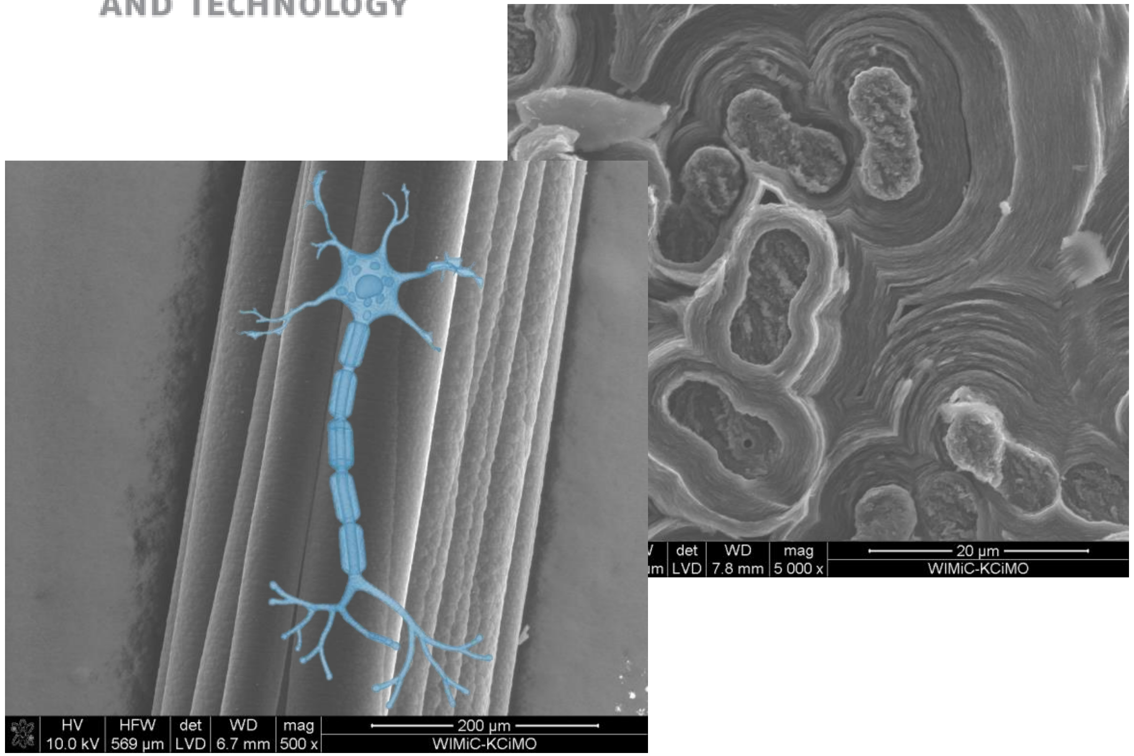

CARBON-CARBON COMPOSITES BASED ON CARBON FIBERS AND PYROLYTIC CARBON AS A POTENTIAL ELECTRODES FOR NERVE CELLS STIMULATION

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BACKGROUND

Parkinson's disease
Neurodegenerative diseases of the nervous system

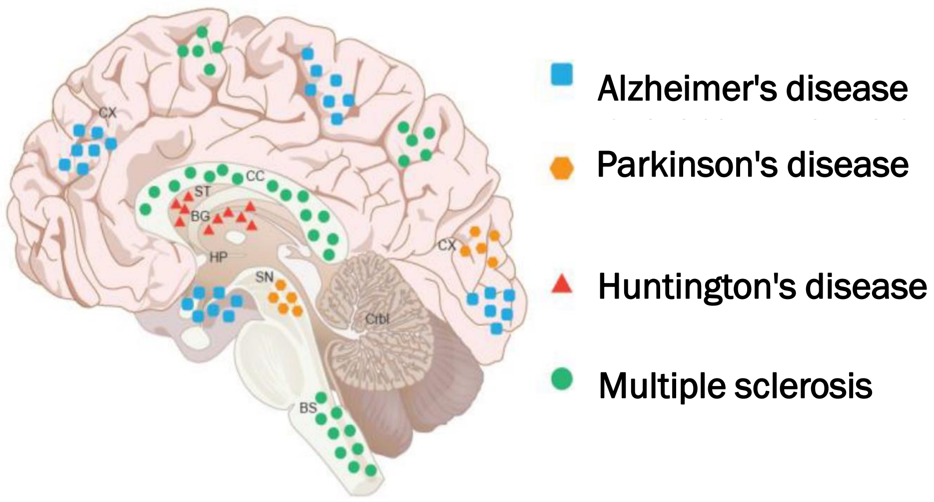
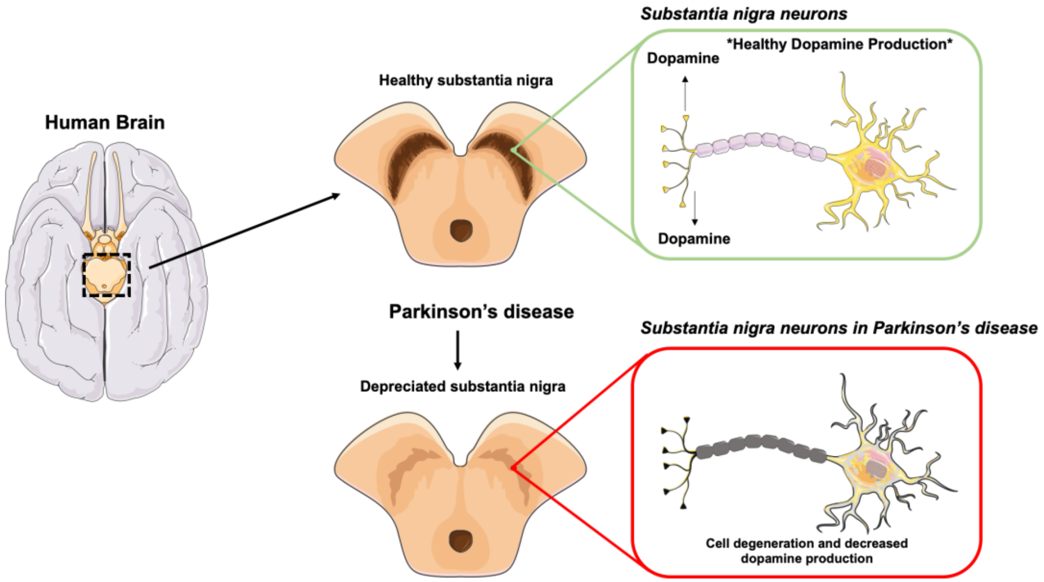


Diagram of the brain with marked areas affected by neurodegenerative diseases

STATISTIC

Global estimates in 2019 showed over 8.5 million individuals with PD. Current estimates suggest that, in 2019, PD resulted in 5.8 million disability-adjusted life years, an increase of 81% since 2000, and caused 329 000 deaths, an increase of over 100% since 2000.

Parkinson disease (PD) is the second most common age-related neurodegenerative deseases diagnosed in North America and Europe. A new study shows the annual incidence of Parkinson's disease (PD) among older adults in the United States is nearly 50 percent higher than the rate currently estimated.



The most noticeable pathological factors in Parkinson's disease are degenerative changes of dopaminergic neurons in the substantia nigra. The loss of dopaminergic neurons that produce dopamine causes a decrease in its level.

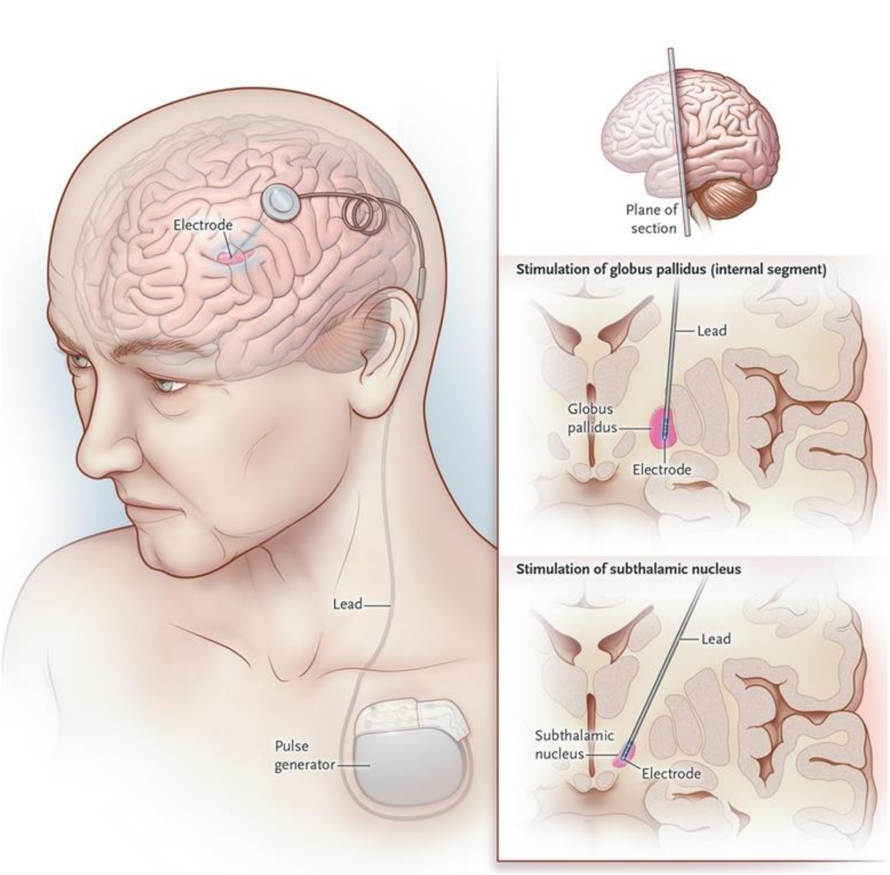
Pharmacological treatments for Parkinson's disease:
- levodopa – a substance that penetrates the brain and is then converted into dopamine
- dopamine antagonists
- MAO inhibitors (monoamine oxidases)
- COMT (catechol-O-methyltransferase) inhibitors
- Anticholinergics
- Amantadine

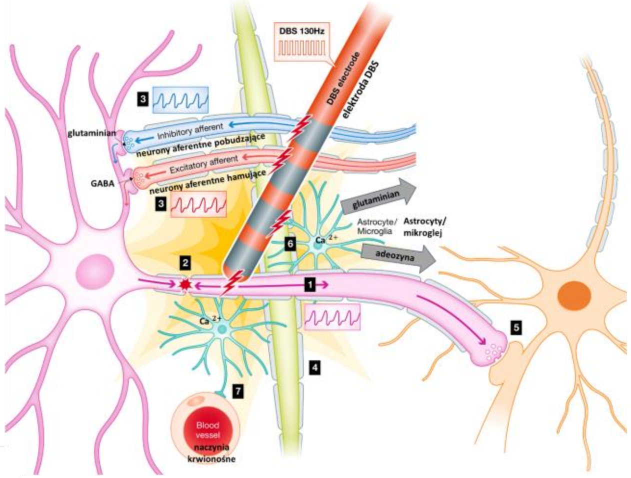
Non-pharmacological methods:
- experimental methods
- deep brain stimulation (DBS)

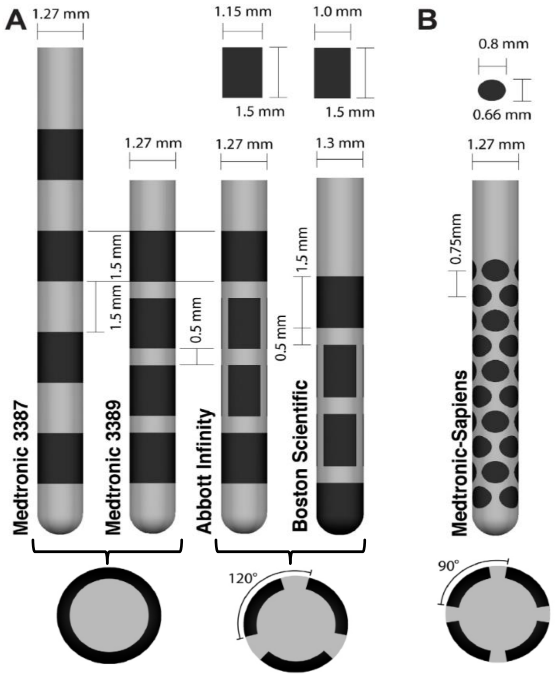
[1] Parkinson's Disease: Is Gene Therapy the Answer We Have Been Looking For? Eoghan J. Mulholland, Ph.D. - April 13, 2020

Metal electrodes are commonly used electrodes in brain stimulation e.g. gold, platinum, iridium, their oxides and alloys (IrOx, PtIr), stainless steel

Deep Brain Stimulation (DBS) electrodes





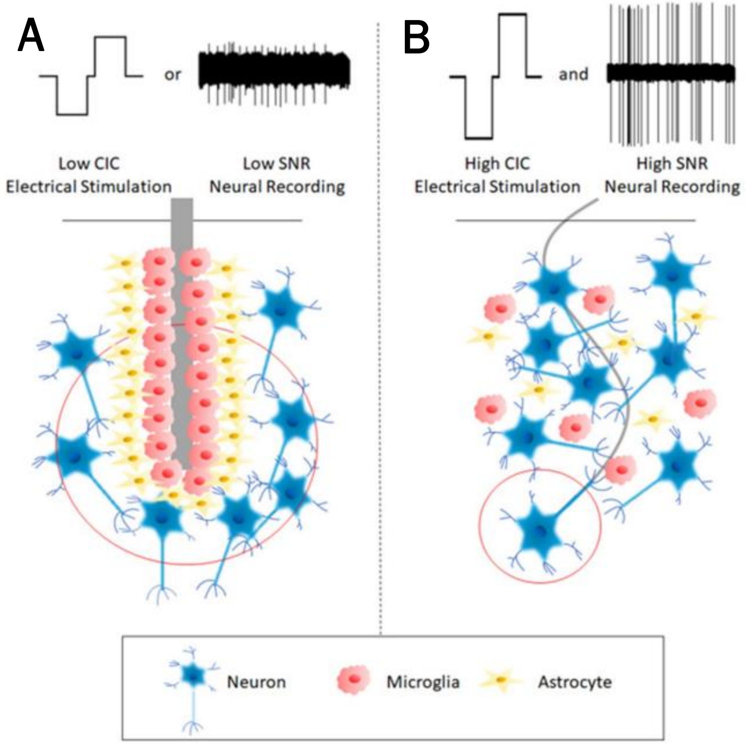


Disadvantages of DBS electrodes:

- Large sizes (diameter 1-3 mm);
- High electricity demand and possibility of brain hemorrhage
- Tendency to corrosion
- High probability of glial scar formation around the electrodes, which increases electrical resistance;
- The stiffness of the electrodes, greater than that of the surrounding tissue, causing tissue destruction.
- Heating during MRI diagnostics

M. S. Okun, „Deep-Brain Stimulation for Parkinson's Disease", *New England Journal of Medicine*, 367 (2012) 1529–1538
S. Zhao *i in.*, „Full activation pattern mapping by simultaneous deep brain stimulation and fMRI with graphene fiber electrodes", *Nat Commun 11 (2020)*
D. N. Anderson, B. Osting, J. Vorwerk, A. D. Dorval, i C. R. Butson, „Optimized programming algorithm for cylindrical and directional deep brain stimulation electrodes", Journal of Neural Engineering, t. 15, nr 2, s. 026005, 2018

Why carbon materials?



A comparison between a conventional implantable electrode (A) and a carbon-based microfiber electrode (B) [1]

Carbon fibers:

- good electrical conductivity (controllability);
- small diameters;
- low density;
- corrosion resistance and flexibility;
- elongated shape;
- wide electrochemical window and electrochemical stability;
- biocompatibility;
- they enable cell differentiation into dopaminergic (mDA) neurons of the midbrain.

Pyrolitic carbon (PyC):

- good electrical conductivity;
- chemical resistance;
- smoothness;
- biocompatibility;
- hemocompatibility.

Carbon nanomaterials:

CNT and graphene

- High electrical conductivity and electrochemical activity;
- High stability during stimulation;
- Ability to induce neuronal differentiation;
- Stimulate the growth of neurites;
- They enhance the electrical signaling of neurons and act as substrates for neuronal growth.

Graphene oxide

- Can promote the differentiation of embryonic stem cells into dopaminergic neurons;
- They stimulate the expression of various integrins responsible for the survival, differentiation and myelination of oligodendrocytes, and promote the differentiation of neurons

[1] Hejazi M, Tong W, Ibbotson MR, Prawer S and Garrett DJ. Advances in Carbon-Based Microfiber Electrodes for Neural Interfacing. Front. Neurosci. 15 (2021) 658703. doi: 10.3389/fnins.2021.658703
[2]Tejchman A., Znój A., Chlebowska P., Frączek-Szczypta A., Majka M. Carbon fibers as a new type of scaffold for midbrain organoid development. International Journal of Molecular Sciences 21 (2020) 1–14.
[3] Frączek-Szczypta A., Jantas D., Ciepiela F., Grzonka J. Graphene oxide-conductive polymer nanocomposite coatings obtained by the EPD method as substrates for neurite outgrowth. Diamond and Related Materials 102 (2020) 1–15
[4] Fraczek-Szczypta A., Jantas D., Ciepiela F., Grzonka J., Bernasik A. Coatings based on graphite oxide and carbon nanotubes for potential application in the regeneration and stimulation of nerve cells, Diamond and Related Materials 84 (2018) 127-140

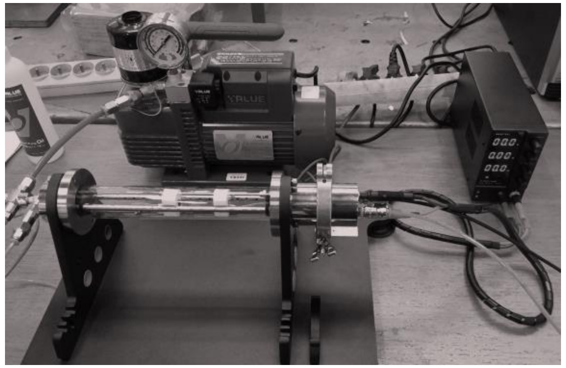
Preparation of C/C composites (carbon electrodes)

Synthesis of C/C composite

Modification of C/C composite using CNT

Preparation of a carbon fiber bundle with a diameter of 50-100µm

- Carbon fibers: high modulus



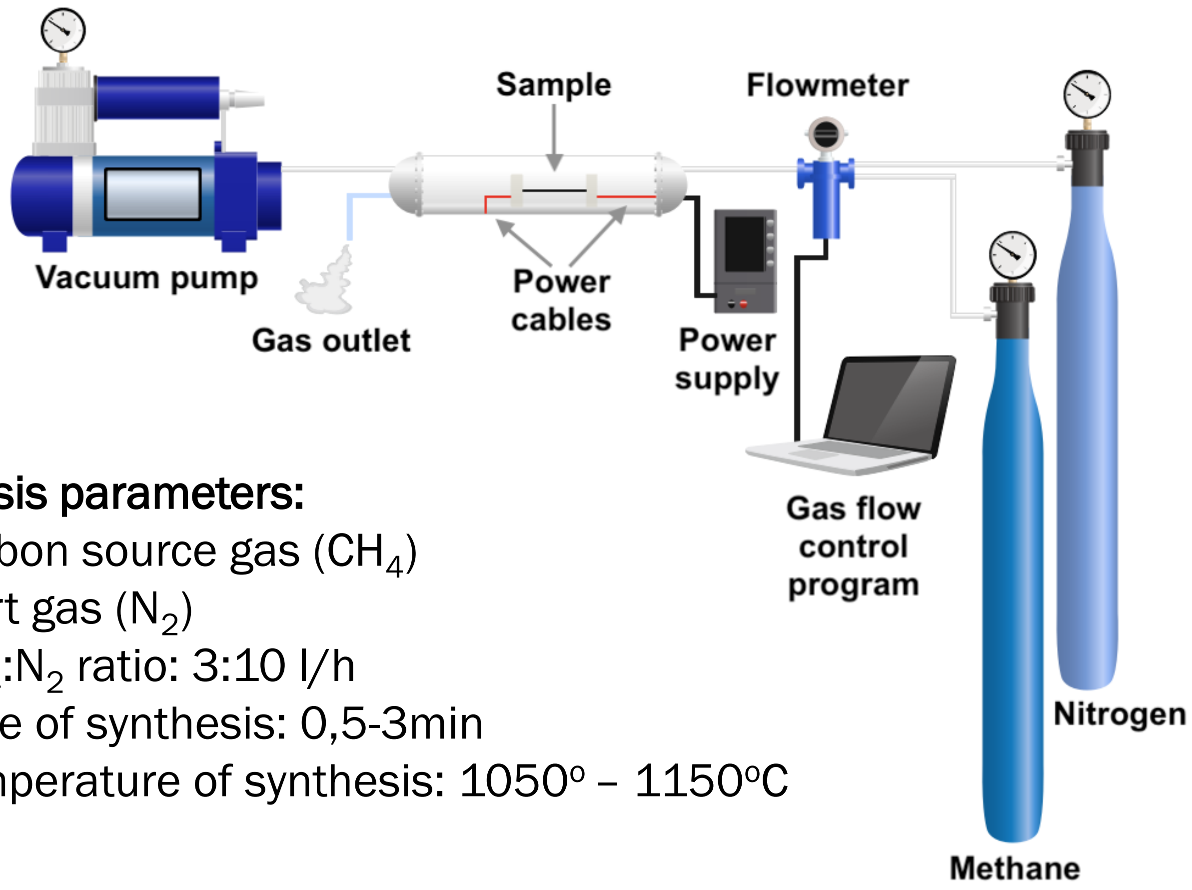
Synthesis of PyC using the CVD method with direct electrical heating of carbon fiber bundles

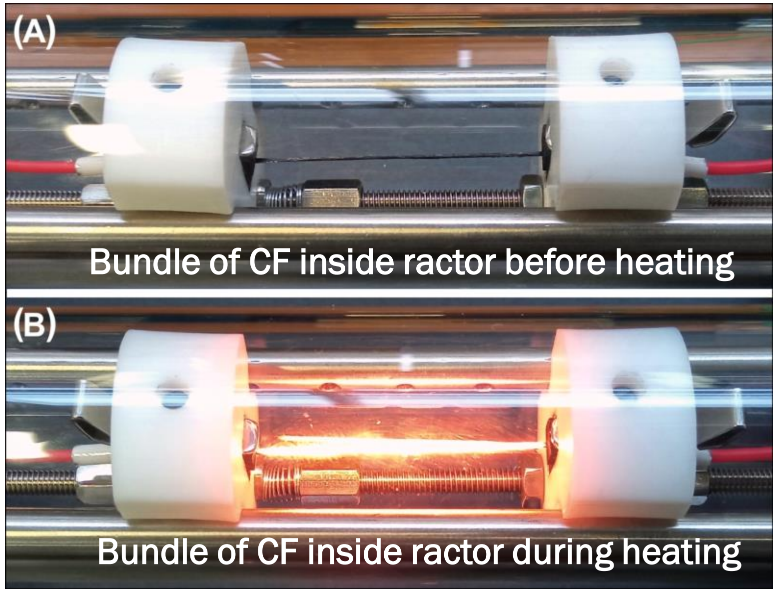
C/C composite less than 1 mm in diameter

Airbrush

C/C hybride composite

Synthesis of C/C composites based on CF and PyC using the CVD method with direct electrical heating



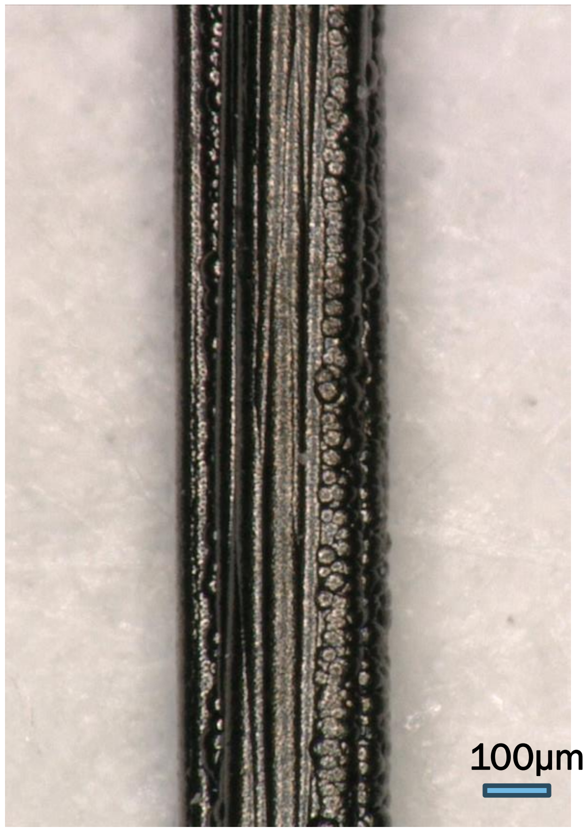


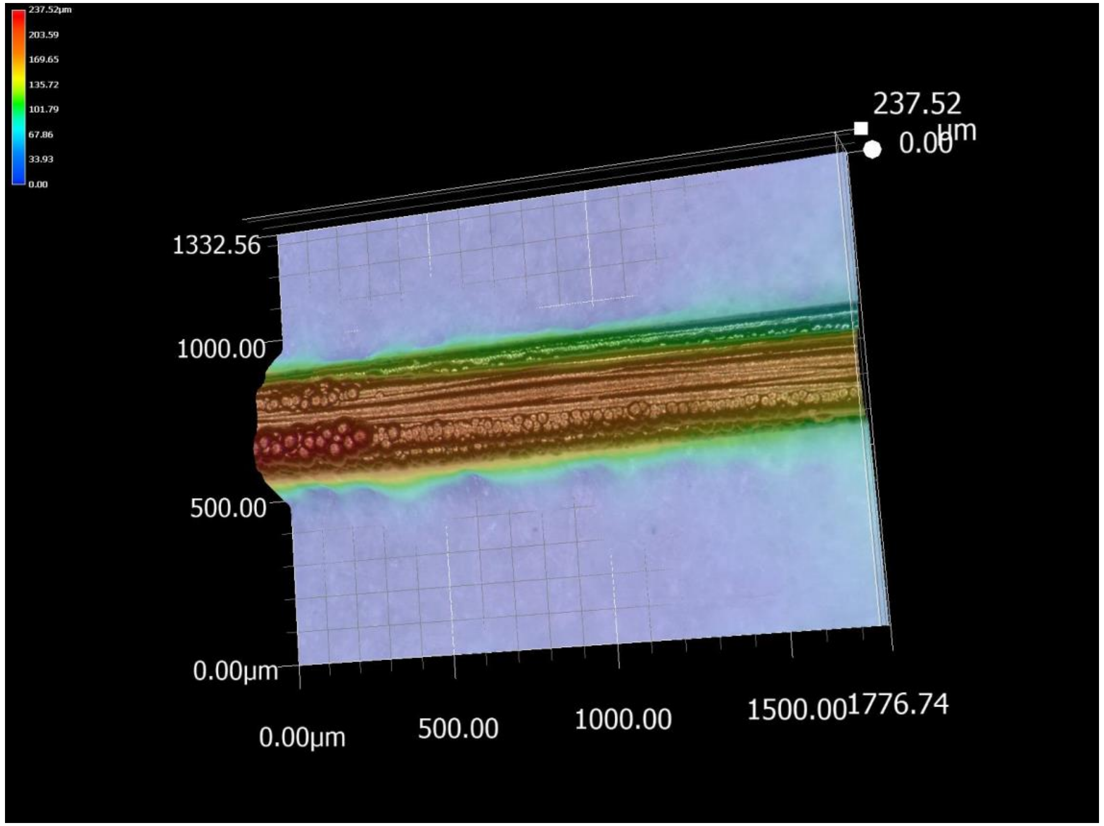
Synthesis parameters:

- Carbon source gas (CH_4)
- Inert gas (N_2)
- CH_4:N_2 ratio: 3:10 l/h
- Time of synthesis: 0,5-3min
- Temperature of synthesis: 1050º – 1150ºC

Total synthesis time, including chamber rinsing and sample cooling: **6-7minutes !**

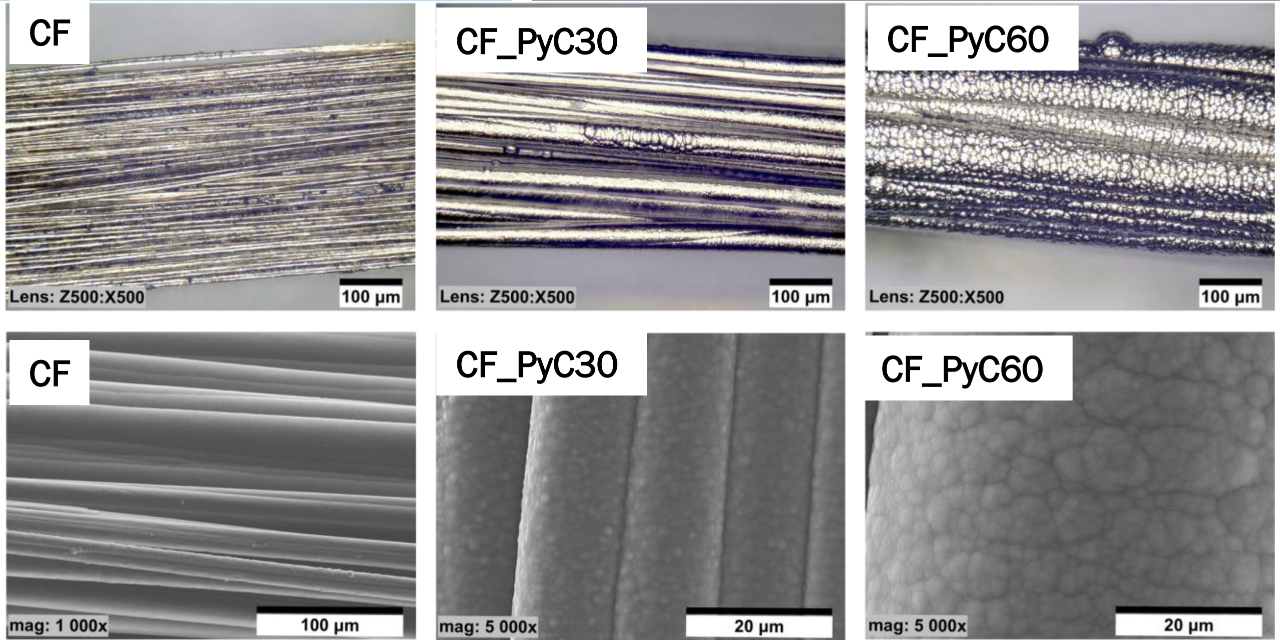
C/C composites (carbon electrodes)

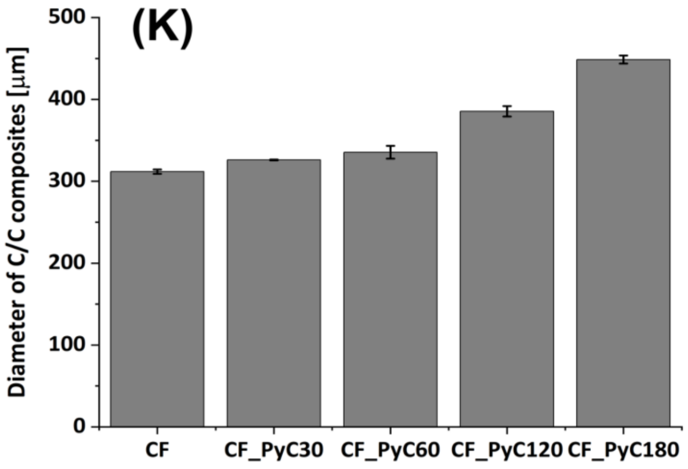


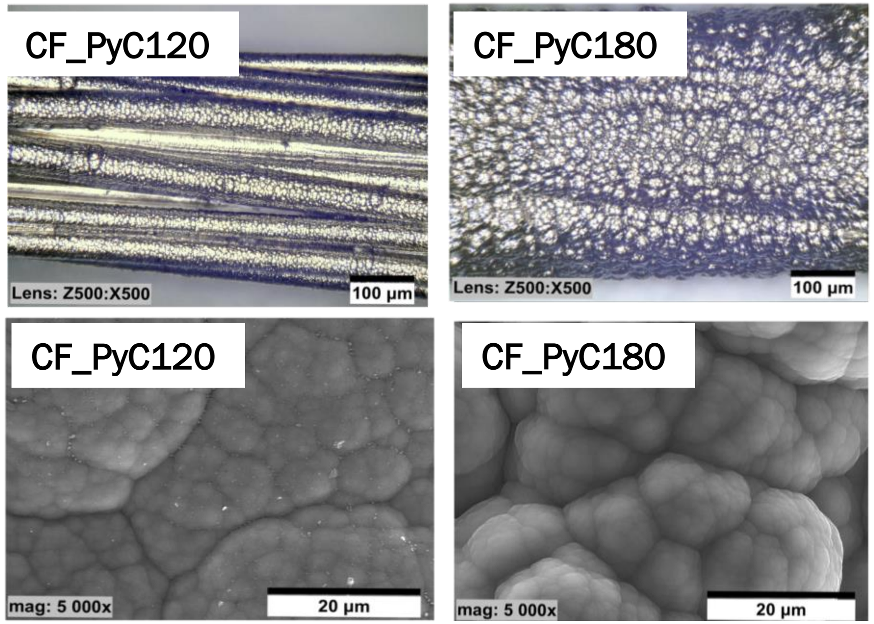


SAMPLES=C/C composites

- **CF_PyC30** – rod-shaped C/C composite based on carbon fiber and PyC obtained after 30s of synthesis.
- **CF_PyC60** - rod-shaped C/C composite based on carbon fiber and PyC obtained after 60s of synthesis.
- **CF_PyC120** - rod-shaped C/C composite based on carbon fiber and PyC obtained after 120s of synthesis.
- **CF_PyC180** - rod-shaped C/C composite based on carbon fiber and PyC obtained after 180s of synthesis.
- **CF** – bundle of carbon fibers





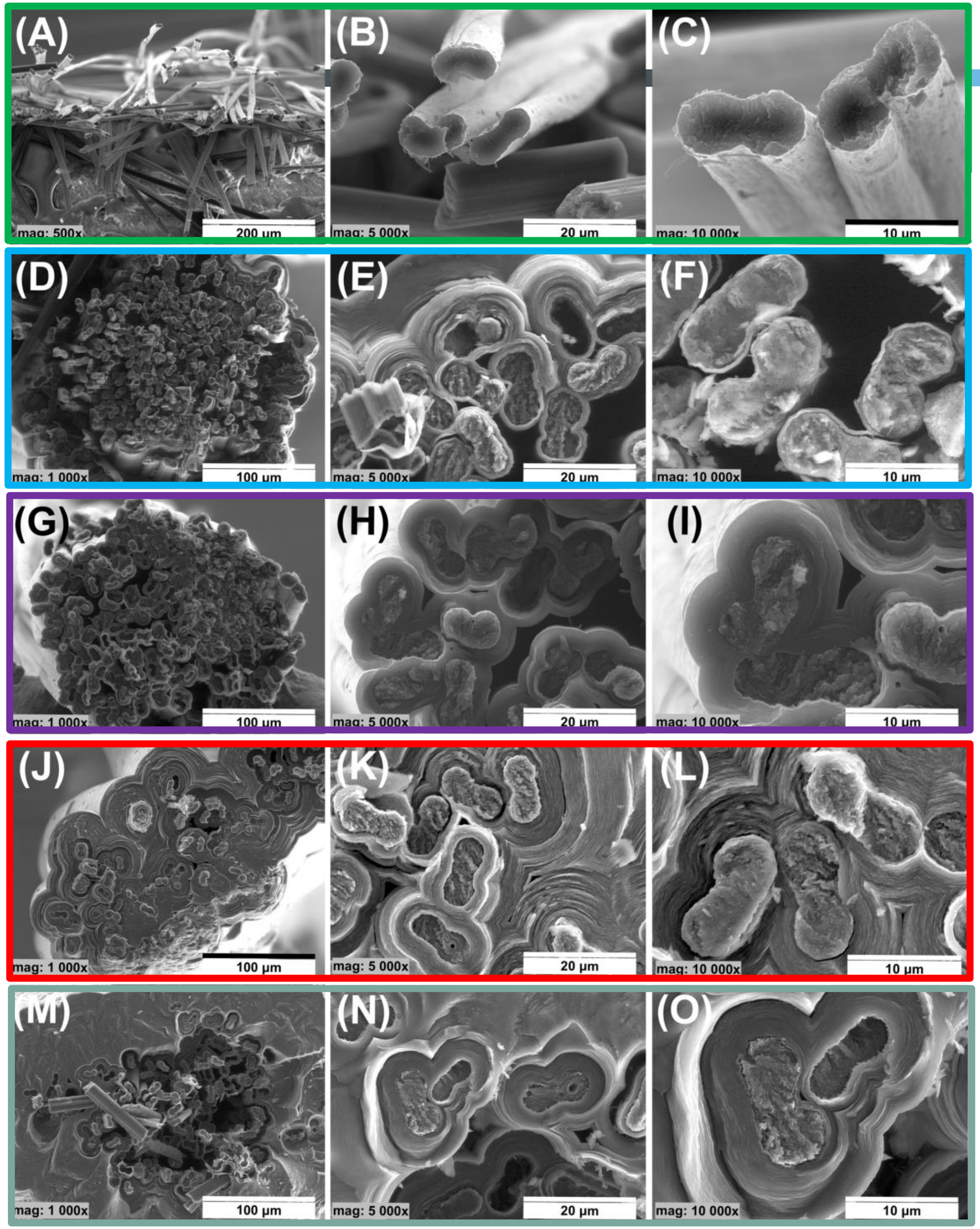


Morphology of a bundle of carbon fibers and C/C composites

A. Fraczek-Szczypta, N. Kondracka, M. Zambrzycki, M. Gubernat, P. Czaja, M. Pawlyta, P. Jelen, R. Wielowski, D. Jantas. *Synthesis of carbon-carbon composites using the CVD method with direct electrical heating of carbon fiber bundles as potential electrode materials for nerve tissue stimulation*. Scientific Report 2023– in press

RESULTS

SEM analysis of cross-sections of the obtained C/C composites depending on the time of PyC synthesis.



CF

Ratio of CF:PyC in composite 80:20

CF_PyC30

Ratio of CF:PyC in composite 60:40

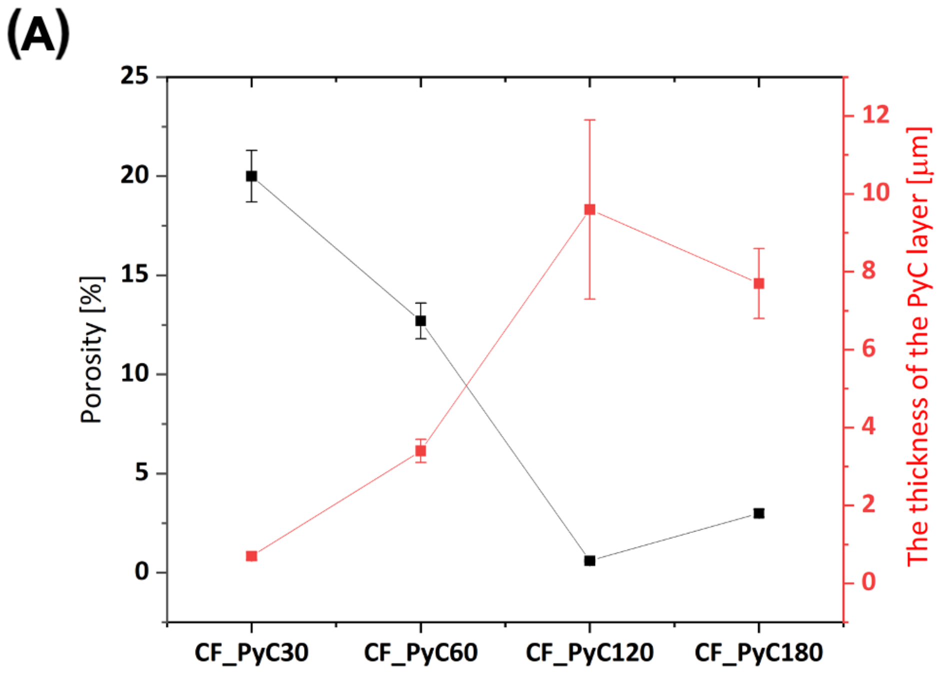
CF_PyC60

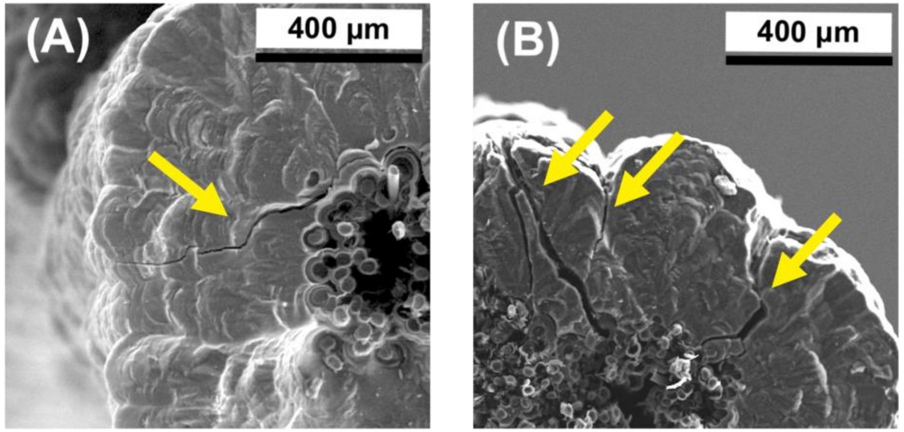
Ratio of CF:PyC in composite 50:50

CF_PyC120

Ratio of CF:PyC in composite 30:70

CF_PyC180



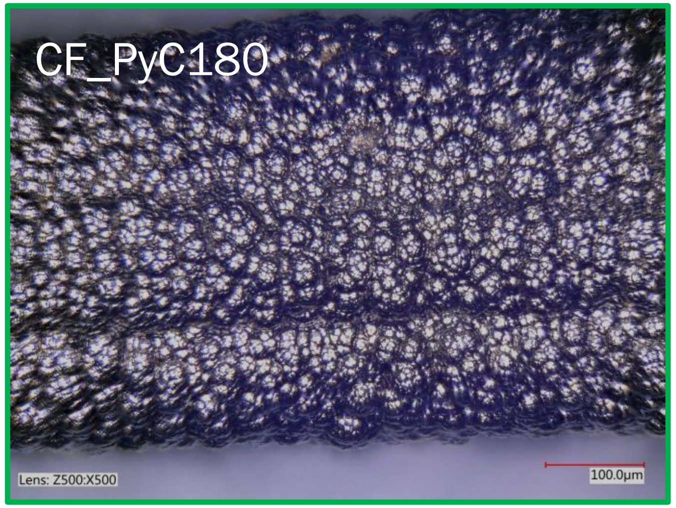


Mechanical properties of C/C composites (static tensile test)

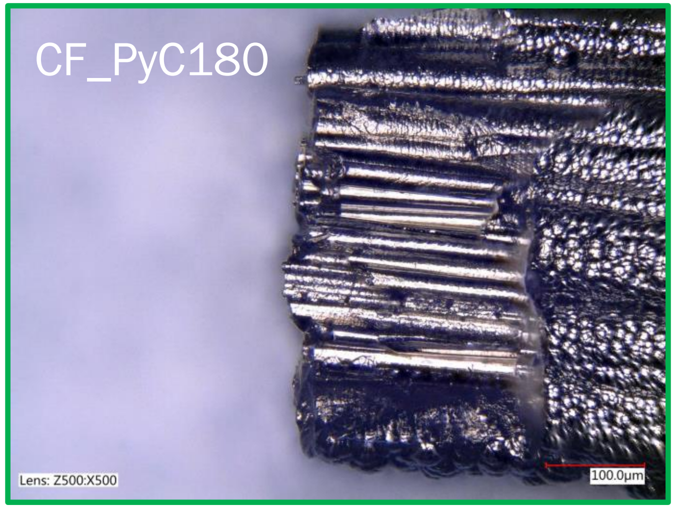
Before

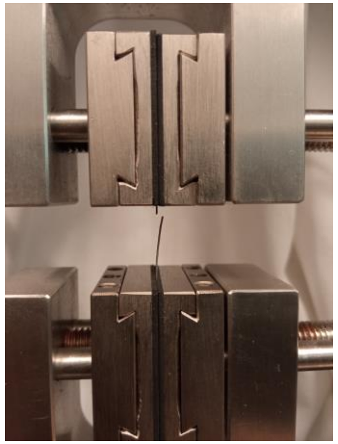
After

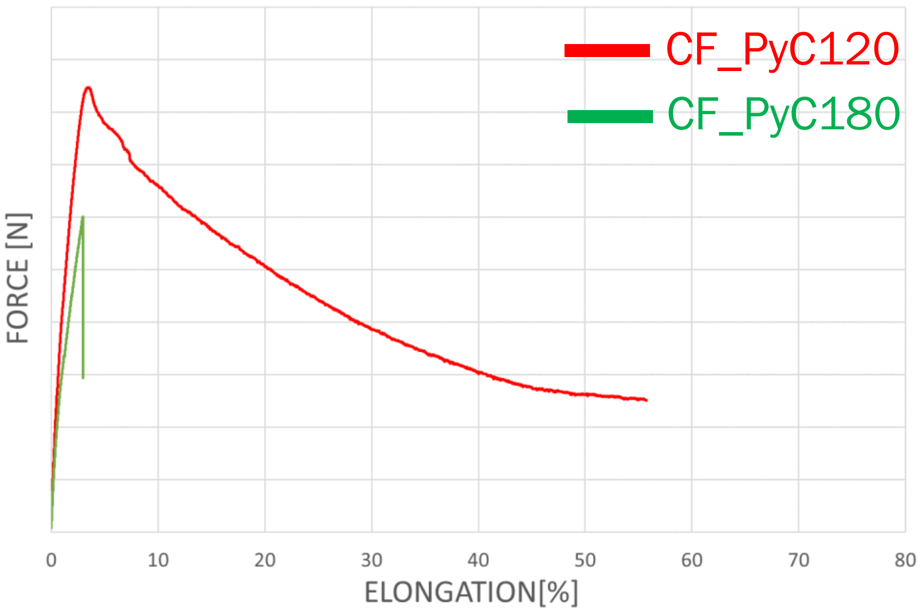
Before



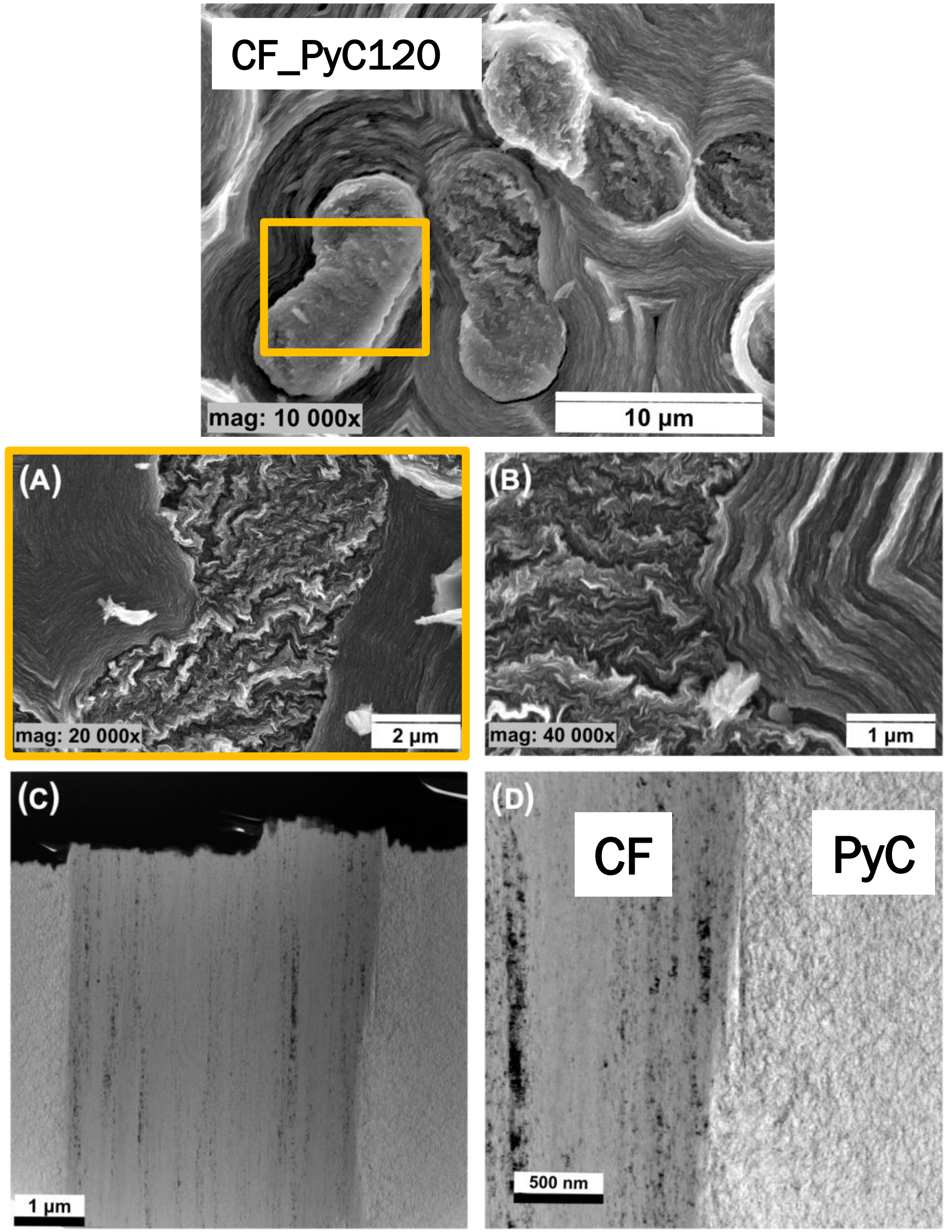
After



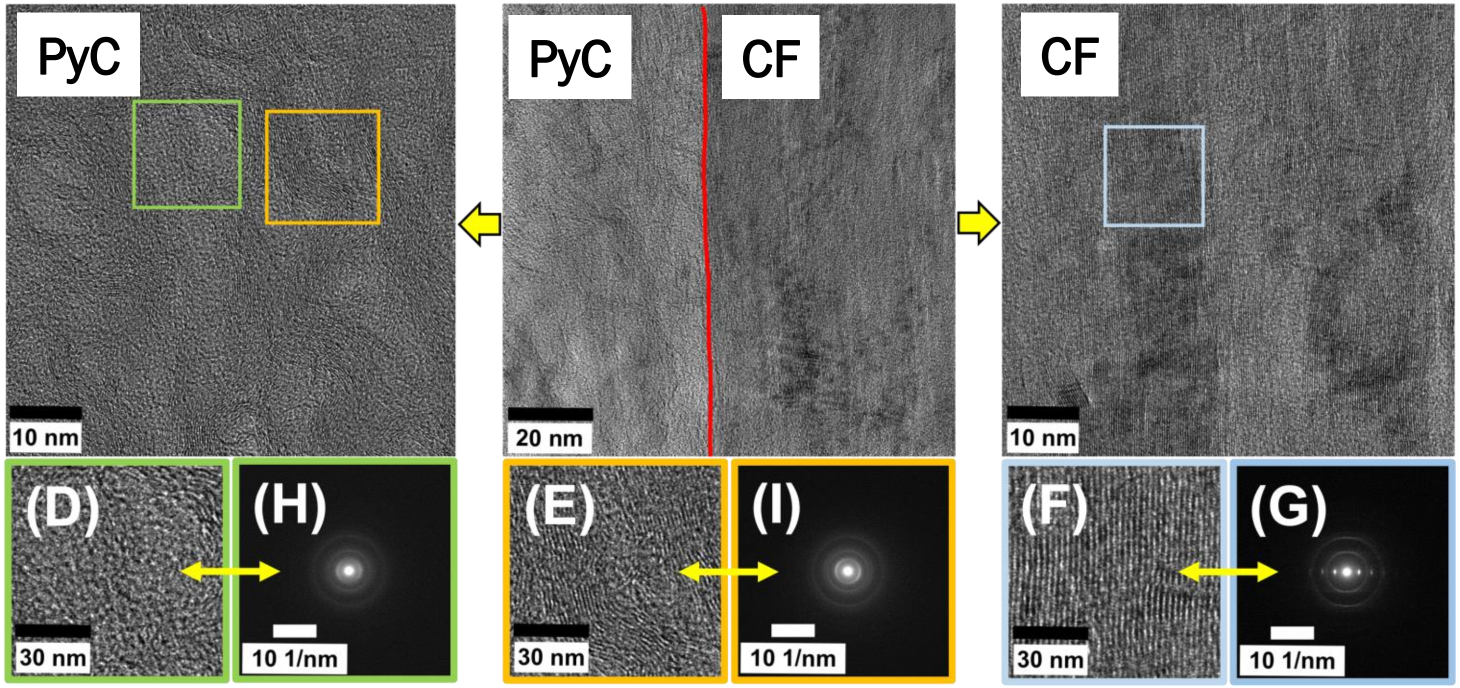




RESULTS



SEM (A,B) and STEM (C,D) morphologies of fracture surface of C/C composites

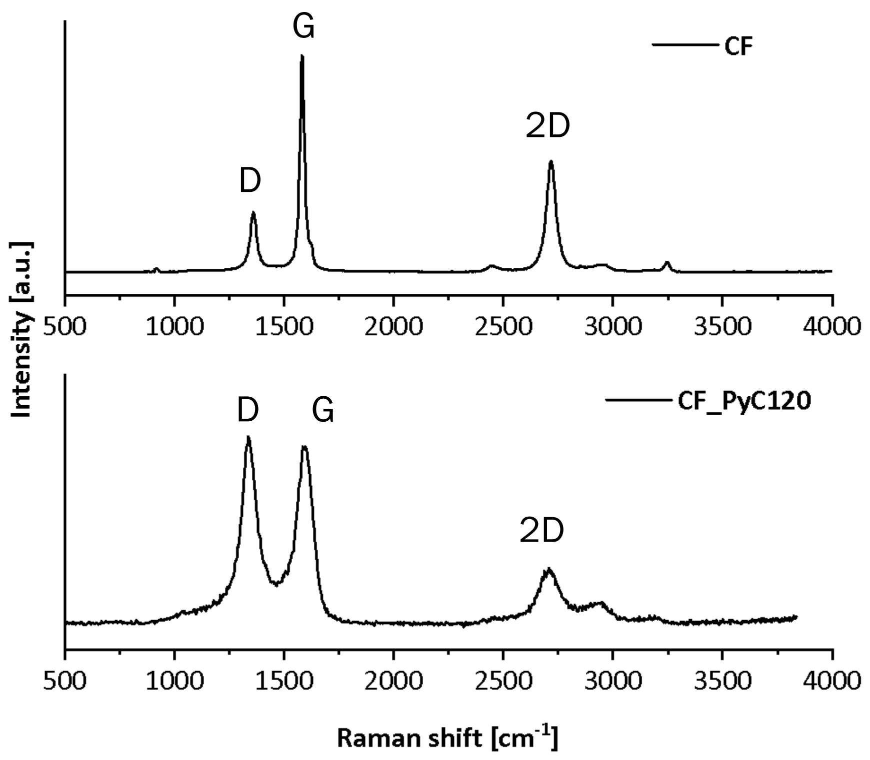


Samples	d_{002} [nm]	L_c [nm]	Orientation angle (OA) [º]
CF	0.339±0.002	8.22±2.80	21
PyC matrix in C/C composite	0.342±0.001	5.94±2.42	76±2 (SL) 93±2 (DL)

The corresponding orientation angles (OA), determined by the selected area electron diffraction (SAED) pattern, indicates the existence of two different textures namely smooth laminar (SL) also called medium textured, OA=76±2º and dark laminar (DL) also called low textured, OA=93±2º. SL pyrocarbon is composed of wavy graphene layers, with strong distortions and curvatures. DL pyrolytic carbon is classified as isotropic carbon, although the preferred orientation of the pyrolytic carbon domains in this type of PyC is between typical isotropic (ISO) and that of typical low-textured ones, i.e. smooth laminar.

RESULTS

Raman spectroscopy

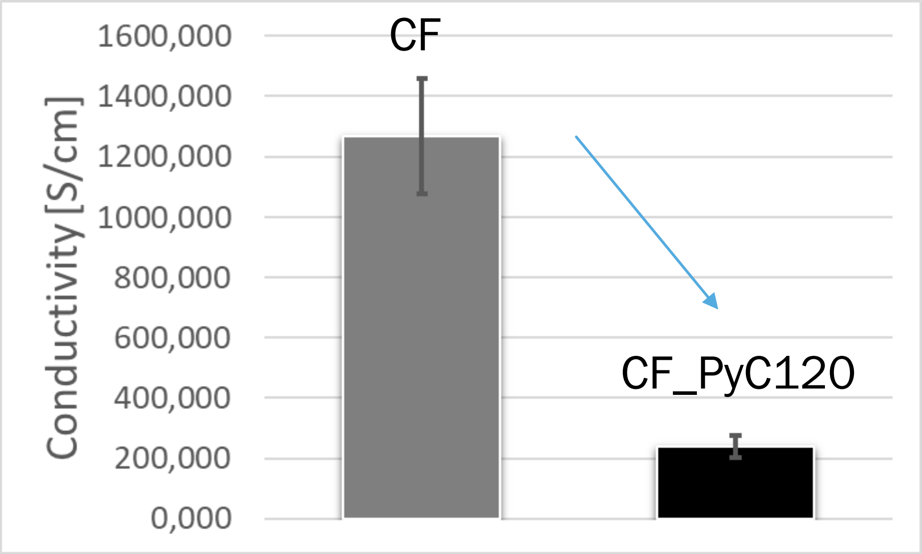


Structural parameters obtained from Raman spectra of CF and CF_PyC120
The values of L_a were obtained from Cancado equation

Sample	I_D	I_G	I_{2D}	I_D/I_G	I_{2D}/I_G	L_a [nm]
CF	972.85	3839.00	1849.72	0.277	0.482	53.71
CF_PyC120	476.2	456.76	141.28	1.064	0.309	13.06

The results of Raman C/C composites studies confirm the results obtained from high-resolution transmission microscopy (HRTEM)

Electrical properties of CF and CF_PyC120



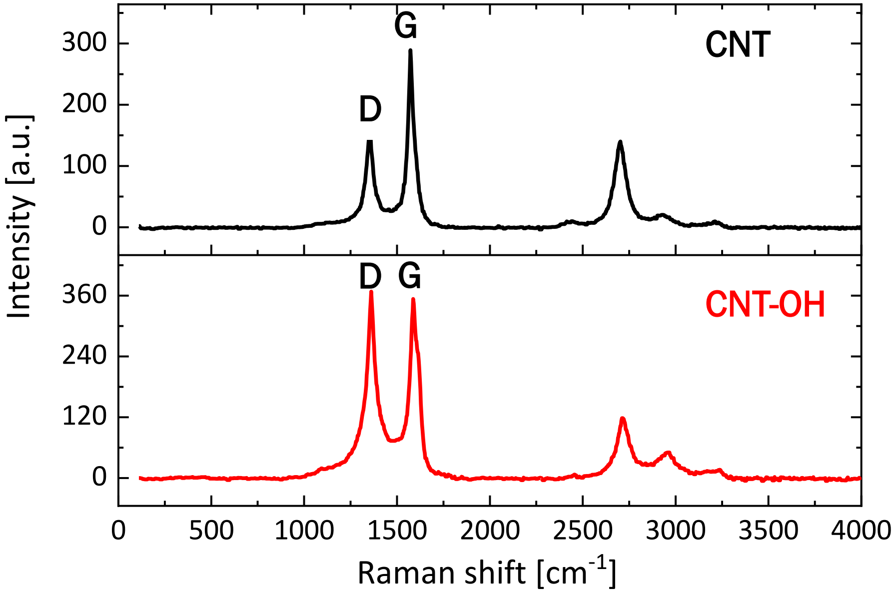
Modification of C/C composite using CNT

C/C composite less than
1 µm in diameter

Airbrush

C/C hybride composite

Raman spectroscopy



Sample	I_D	I_G	I_D/I_G
CNT	155.7	289.1	0.538
CNT-OH	367.9	353.4	1.041

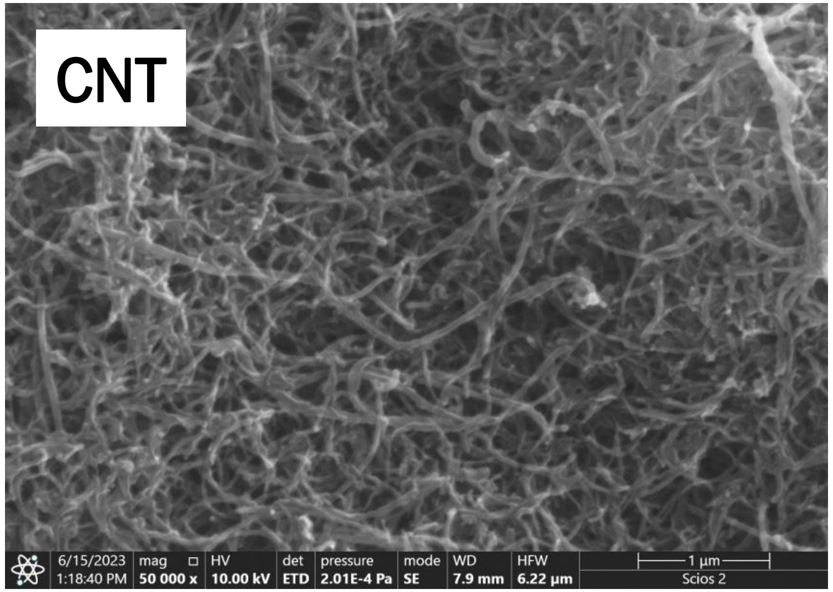
BET surface area

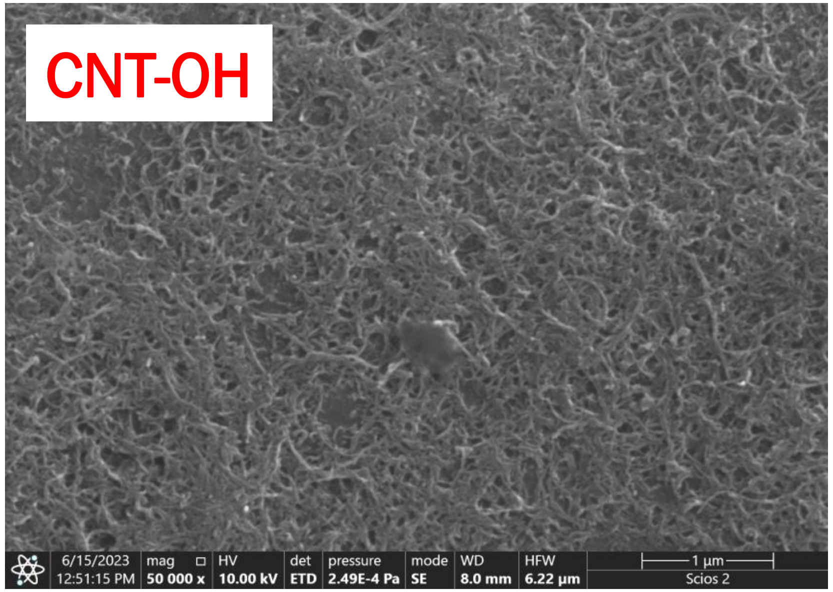
Sample	BET [m^2/g]
CNT	84.0
CNT-OH	508.3

Dimention of CNT

Sample	Diameter [nm]	Length [µm]
CNT	10-20	0.5-2
CNT-OH	10-20	<1

SEM



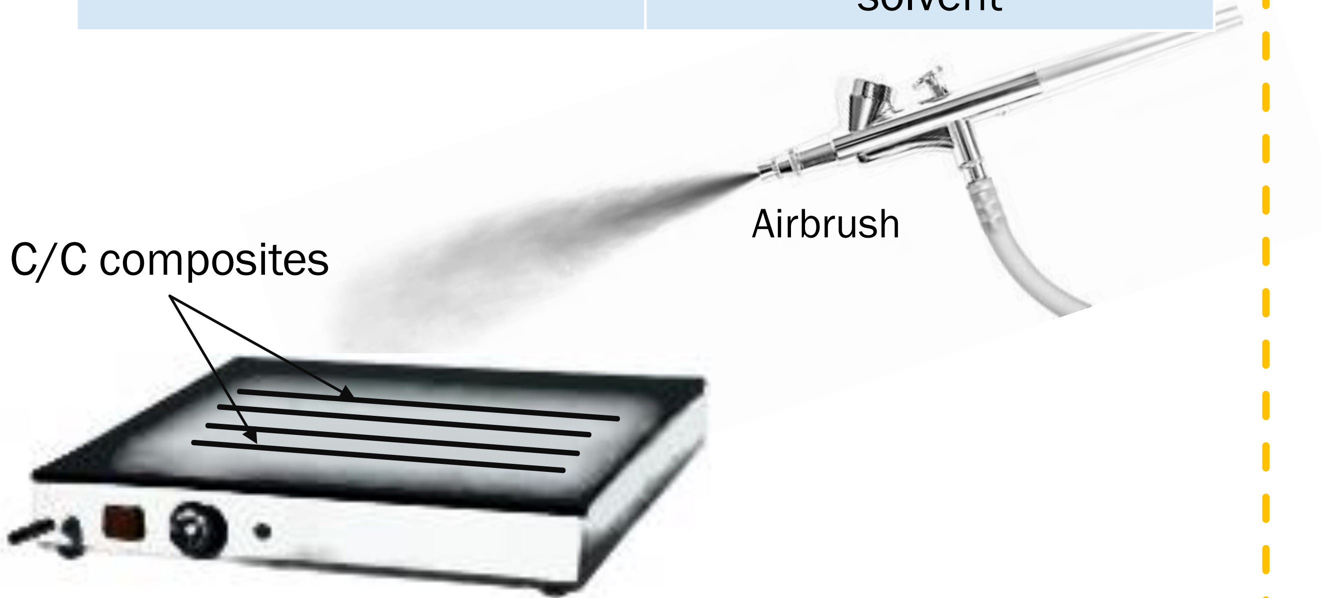


Carbon nanotubes (CNT)

RESULTS

Modification of C/C composites with CNT

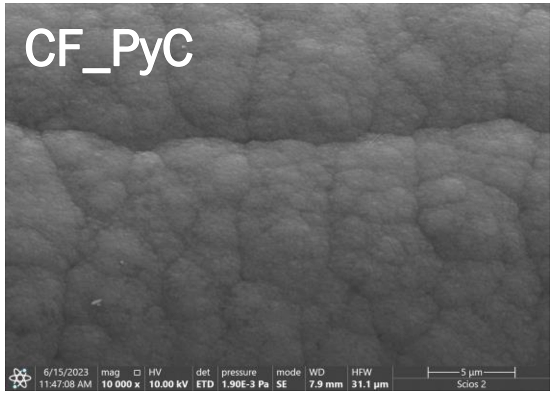
CNT	CNT-OH
3mg/ml MWCNT+isopropanol	3mg/ml MWCNT-OH+mixture of solvent

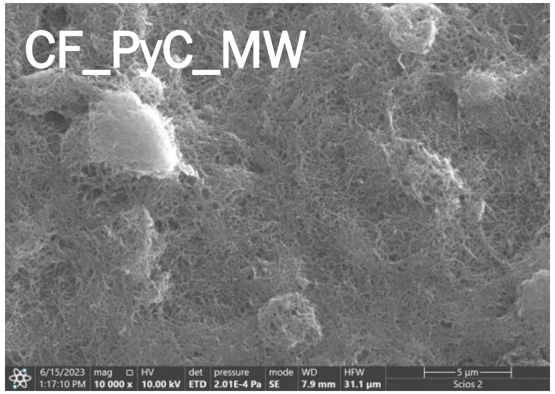


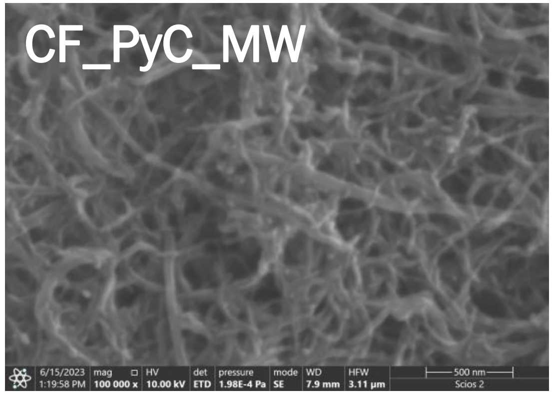
Process condition:

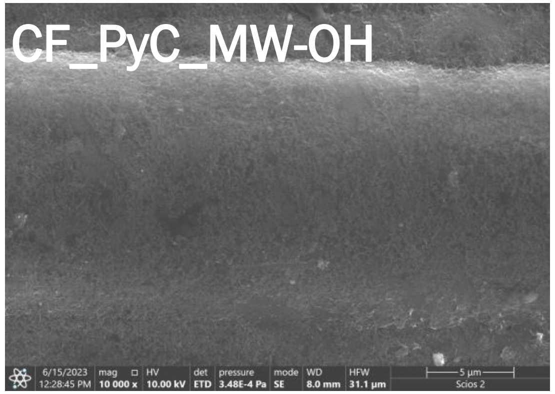
- CNT concentration: 3mg/ml
- Time: 150s/side
- Flow rate: 0.33ml/min
- Amount of solution 0.20-0.50 ml/side
- Compressor pressure: 1.2-1.3bar
- Heating plate temperature: 225°C

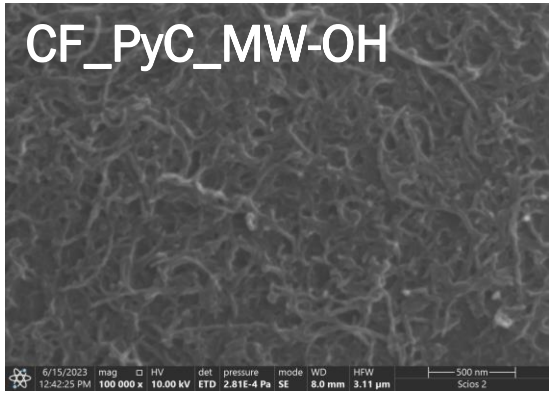
Surface morphology







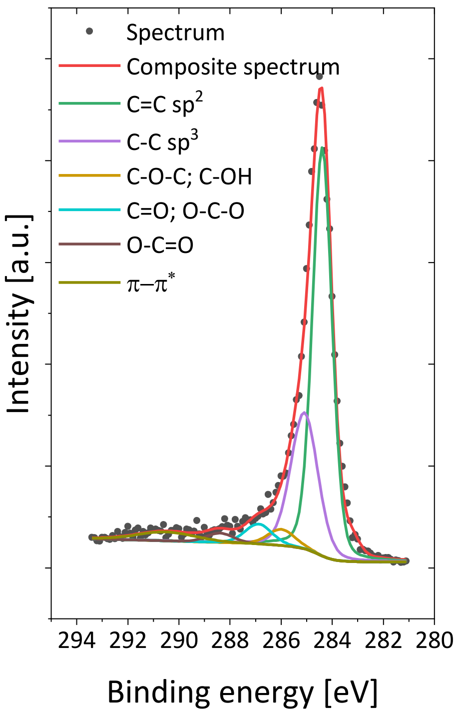




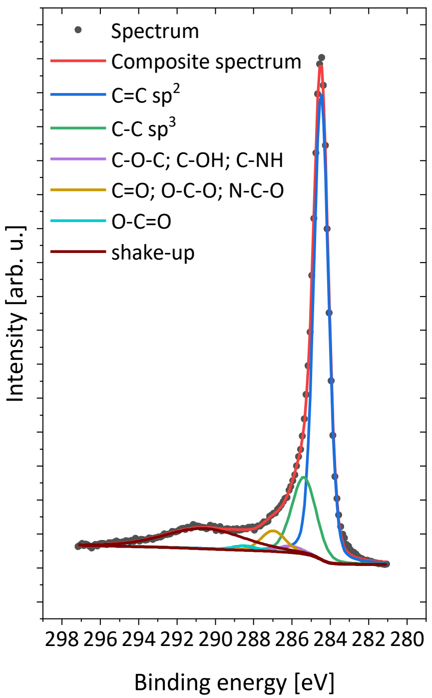
RESULTS

Sample	Elemental composition [%]		[-]	Deconvolution of the C1s spectra [%]					
	C	O	O/C	284.5eV C=C (sp^2)	285.3eV C-C (sp^3)	286.1eV C-O, C-OH	287.0eV C=O, O-C-O	288.5eV O-C=O	291.0eV $\pi \rightarrow \pi^*$
CF_PyC	90.30	9.70	0.11	56.10	23.10	2.80	3.40	1.60	3.30
CF_PyC_CNT	98.20	1.60	0.02	60.50	16.50	1.40	4.50	1.00	14.30
CF_PyC_CNT-OH	85.70	12.8	0.15	42.50	23.20	7.40	3.00	4.60	5.00

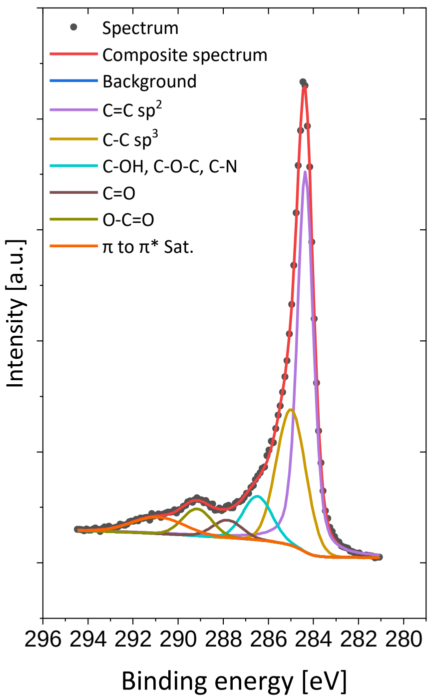
CF_PyC



CF_PyC_MW



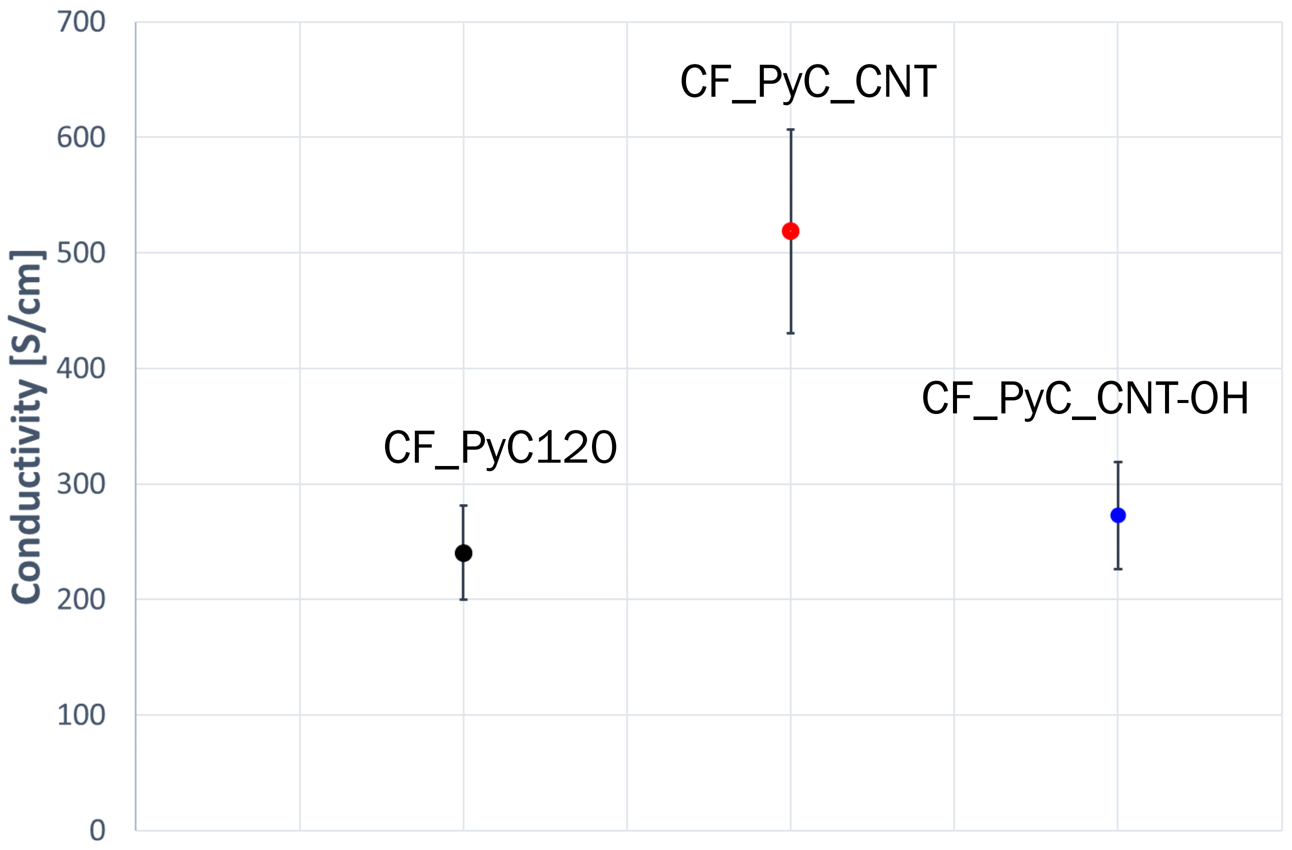
CF_PyC_MW-OH



Sample	Water contact θ_{adv} [º]
CF_PyC	**88.58±2.17**
CF_PyC_CNT	**111.24±1.61**
CF_PyC_CNT-OH	**47.50±1.35**

- The share of CNT without functionalization causes a decrease in the amount of oxygen groups and an increase in the hydrophobicity of the composites
- The presence of CNTs after functionalization significantly decreases the hydrophobicity of composites

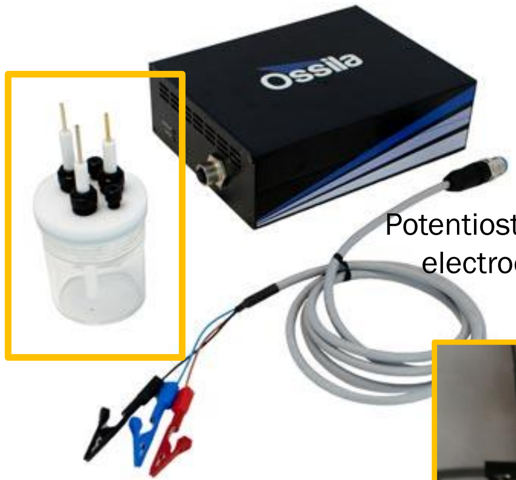
Electrical properties of CF_PyC120 before and after modification using CNT



- Electrical properties of CF_PyC composites increase after modification of CNT without modification;
- Presence of functionalized CNT does not change the electrical properties of the C/C composite compared to the unmodified one.

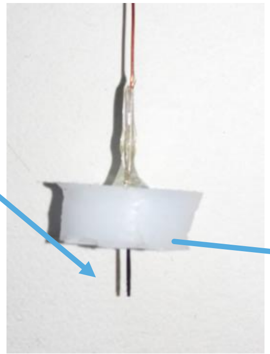
RESULTS

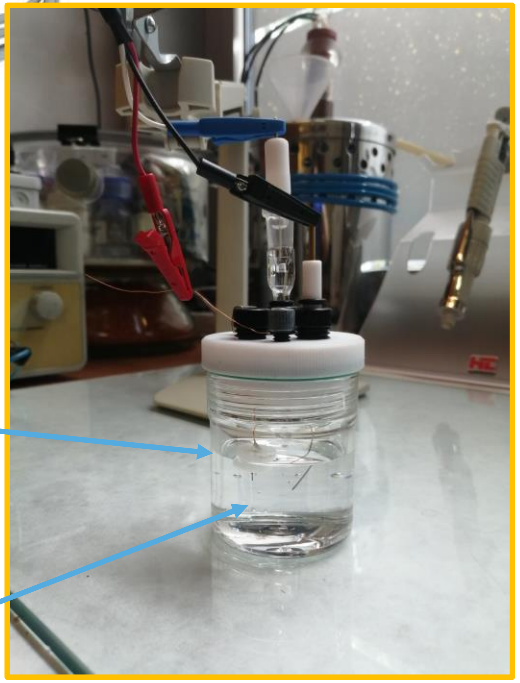
Cyclic voltammetry (CV)



Potentiostat with cells and electrodes Ossila Co.

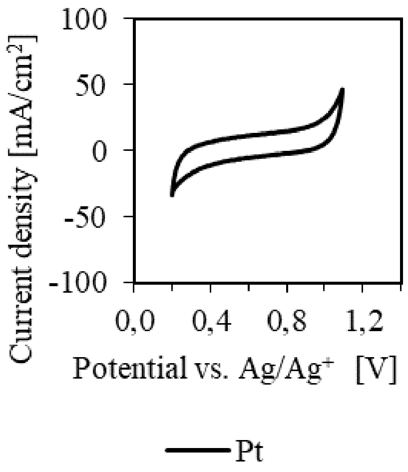
Working electrodes

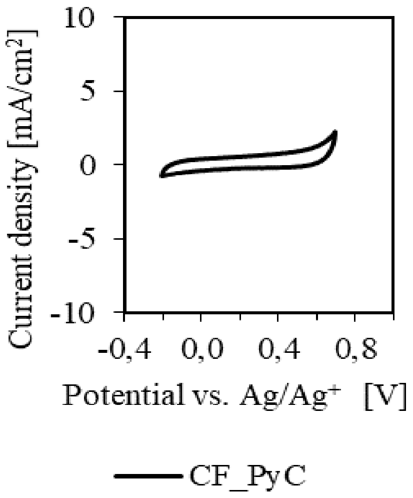


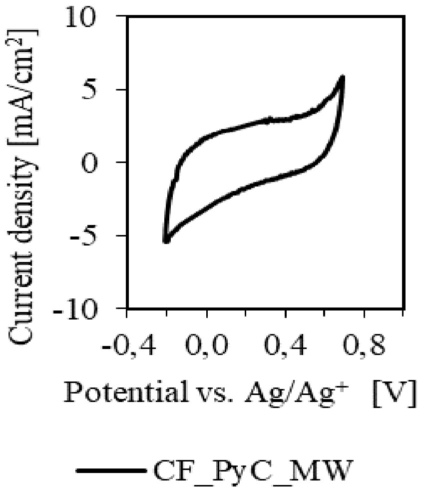


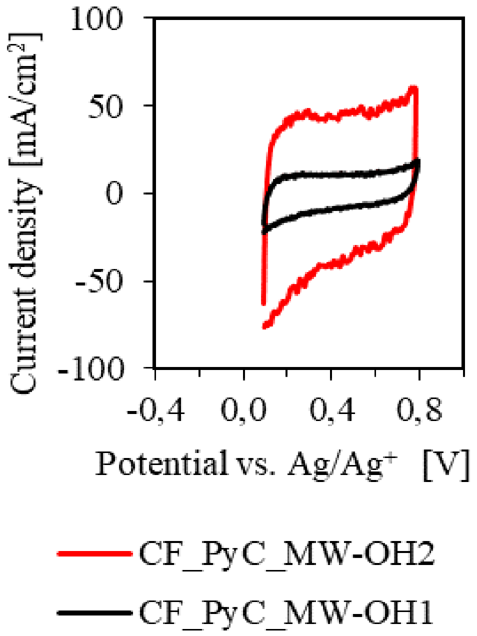
Artificial cerebrospinal fluid (aCSF)

Electrochemical cell





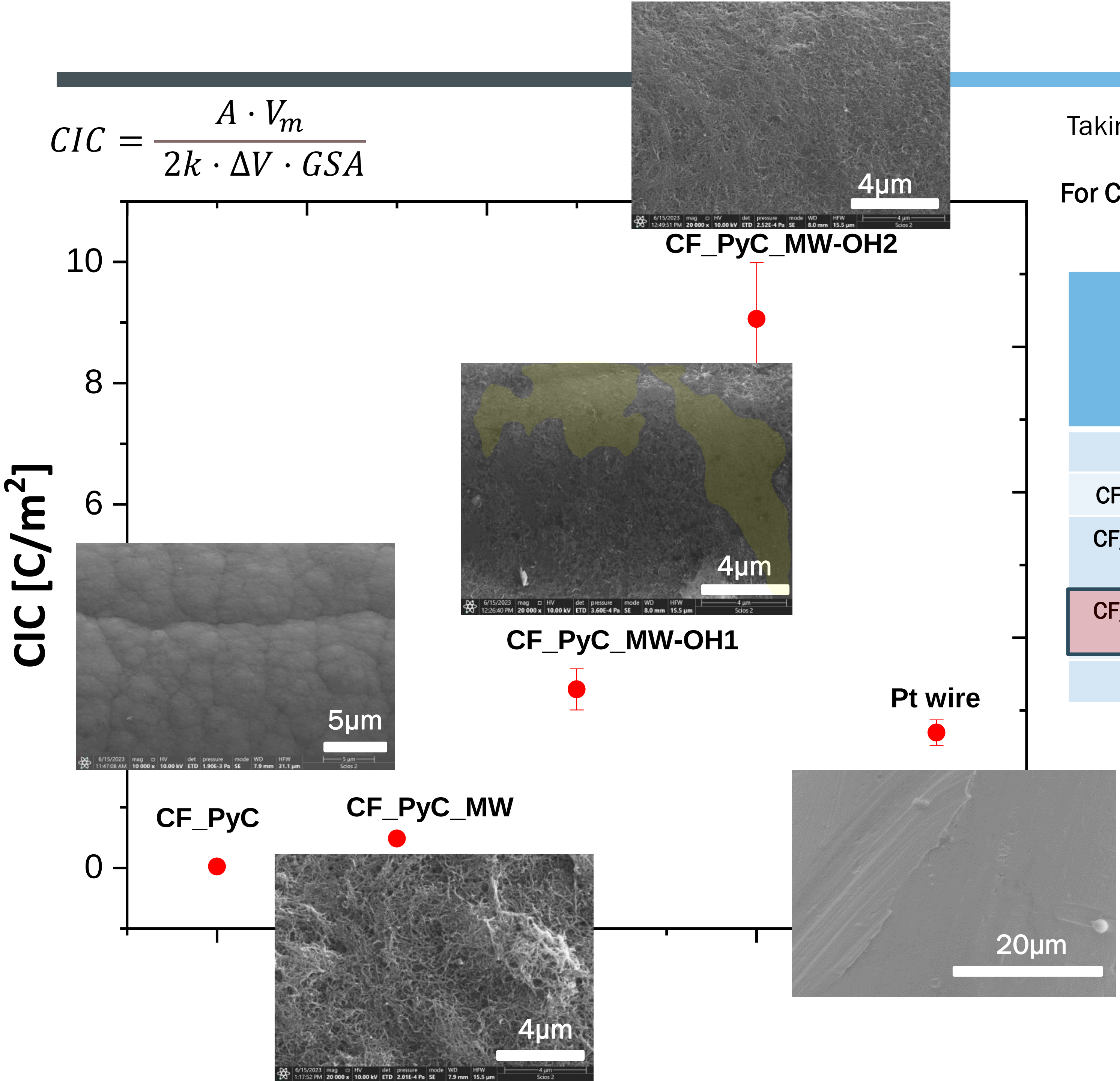




- The voltammograms obtained for the scanning rate of 50 mV/s are shown in the graphs
- Carbon-carbon composites (CF_PyC) was the lowest surface area in compasion with rest of samples.
- Modification of CF_PyC surface using CNT increases the surface area of electrodes in compart to pure CF_PyC.
- **The largest surface area has CF_PyC_MW-OH2 composites, it is almost 9 times higher than the area for Pt.**

RESULTS

$$CIC = \frac{A \cdot V_m}{2k \cdot \Delta V \cdot GSA}$$



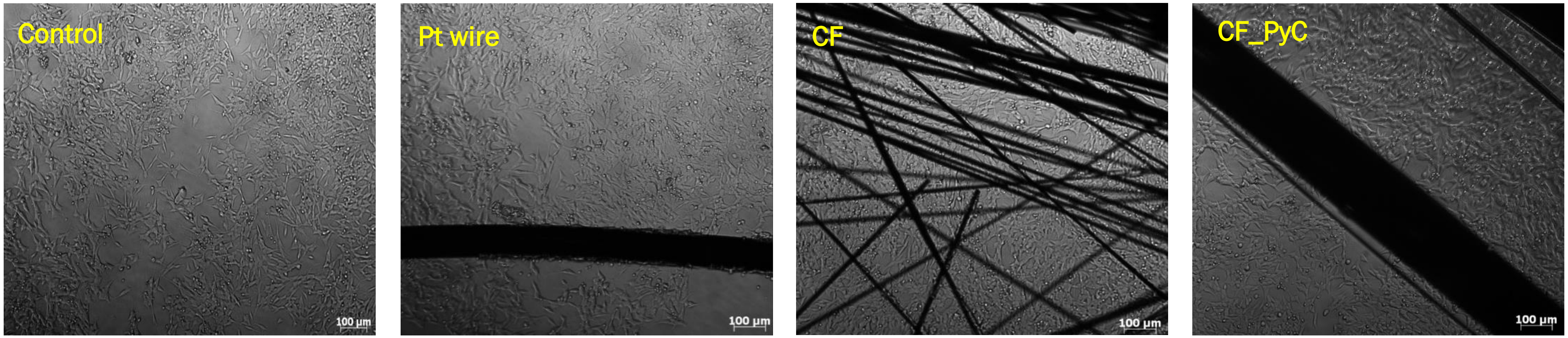
Taking into account potential range, area and GSA, charge injection capacity (CIC) values were calculated.

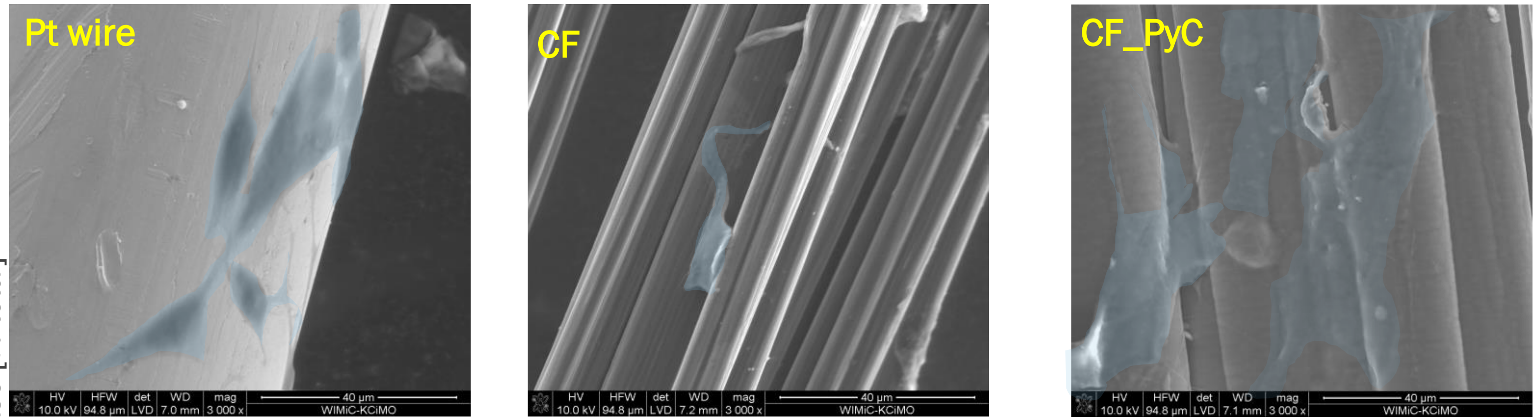
For CF_PyC_MW-OH2, the value is almost 5 times higher than for Pt.

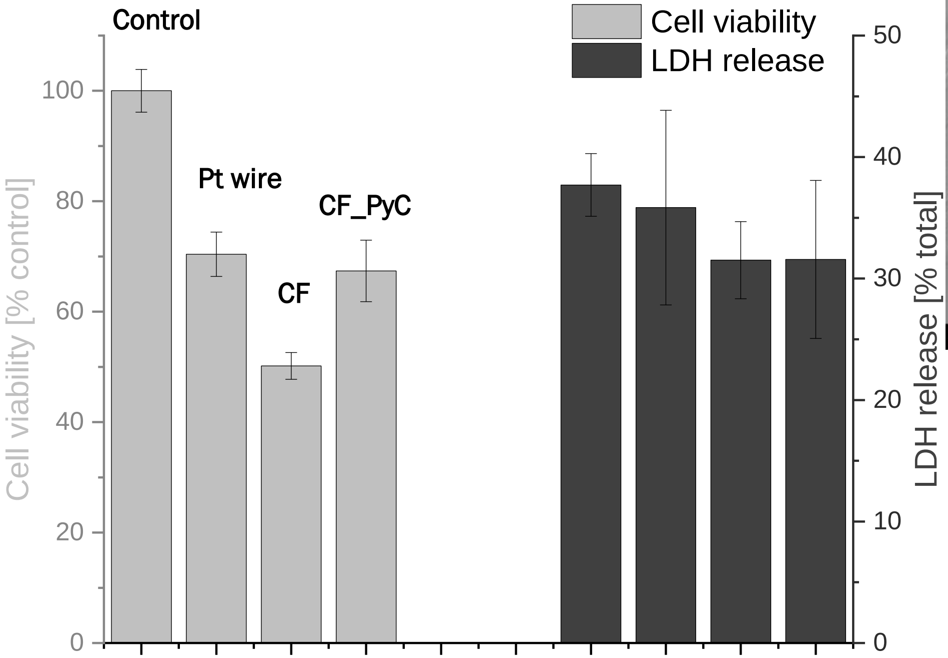
Sample	GSA [mm^2]	CIC [C/m^2]	Concentration MWCNT on electrode surface [mg/mm^2]	Water window [V]
CF_PyC	5.34	0.1033	-	-0.2-0.7
CF_PyC_CNT	3.43	0.4847	0.20	-0.2-0.7
CF_PyC_CNT-OH1	3.64	2.265	0.30	0.1-0.8
CF_PyC_CNT-OH2	3.30	9.988	0.70	0.1-0.8
Pt	1.42	2.085	-	0.2-1.1

The increase of the CIC parameter for CF_PyC_CNT-OH2 is probably related to the higher content of MWCNT-OH forming a homogeneous layer on the surface of the carbon electrode and the presence of functional groups affecting the growth of electrochemical active surface area of electrode (ECSA).

Human neuroblastoma cell line SH-SY5Y



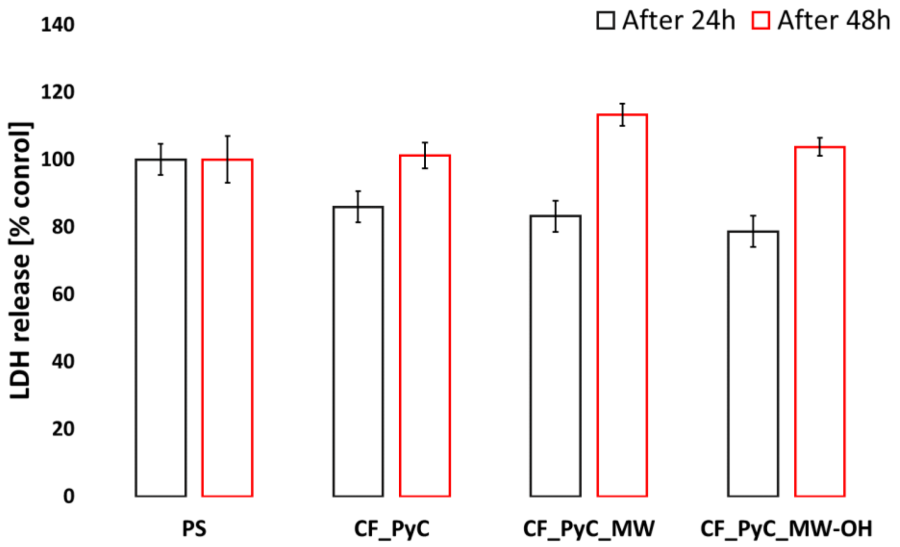




- No cytotoxic effect was observed in any of the analyzed samples.
- Cell viability on the C-C composite samples is at the Pt wire level and was higher than on the CF sample.

Preliminary biocompatibility study of C-C composites after modification

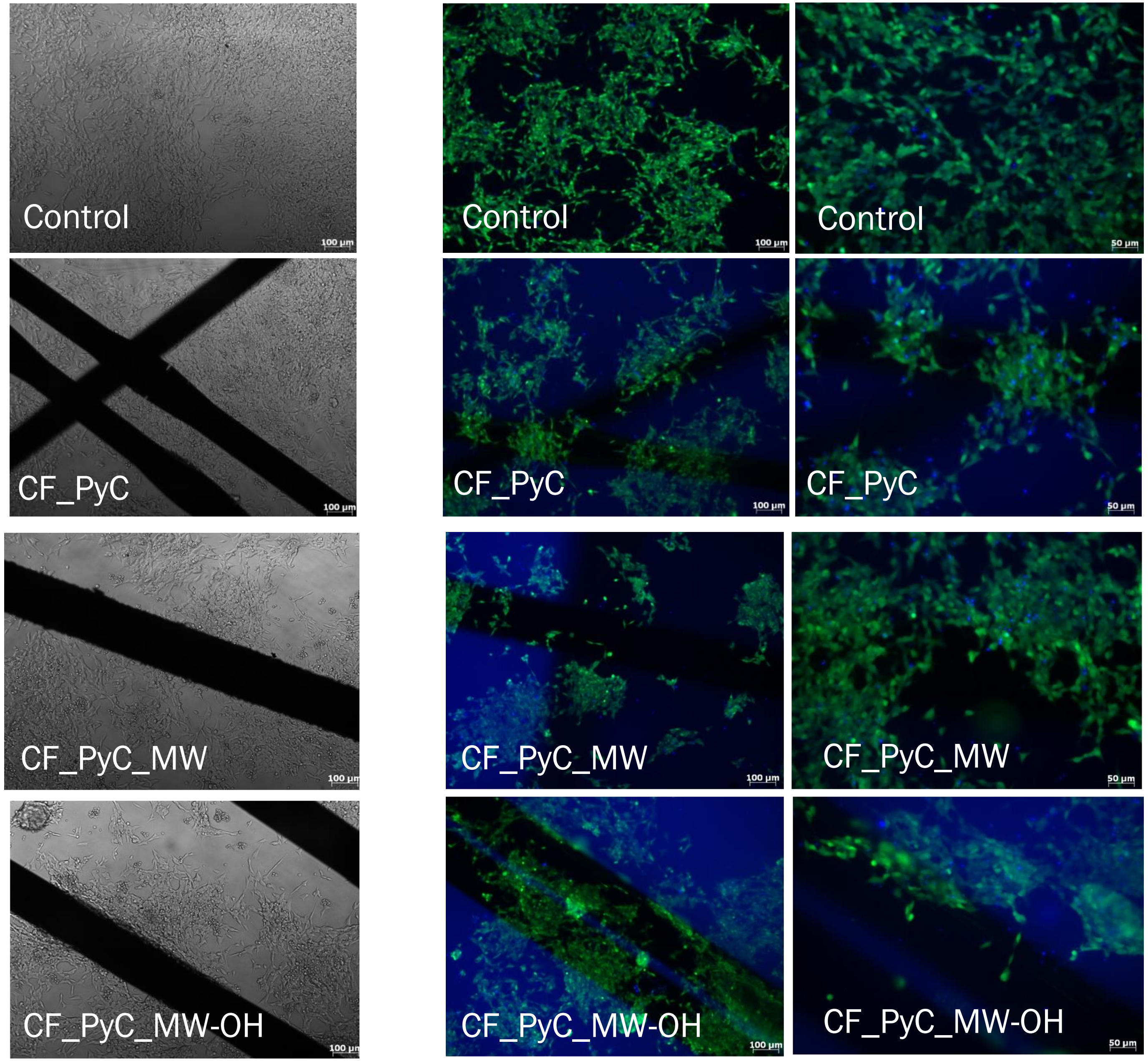
Human neuroblastoma cell line SH-SY5Y



- No cytotoxic effect was observed in any of the analyzed samples both before and after modification.
- Qualitative analysis of cell viability showed no clear differences in morphology between the cells in the control sample and the test samples.
- Surface modification of CF_PyC with CNT did not affect cell morphology compared to the pristine sample
- In the case of CF_PyC_MW-OH composites, some differences in the behavior of the cells in direct contact with the material can be observed. In this sample, the cells adhere more clearly to the surface of the carbon electrode.

Differential interference contrast (DIC)

CalceinAM/Hoechst 33342 double staining and fluorescence imaging



CONCLUSIONS

- The CVD method with direct heating of the sample allows to obtain a C-C composite with diameter significantly below 1mm (average 200-300μm);
- The presence of PyC increases the shape stability of the fibers and their handiness
- By controlling the process parameters, such as the synthesis time, we can control the thickness of the pyrolytic layer what have influence on mechanical properties of C/C composites
- The presence of PyC causes a decrease in the electrical conductivity of the composite compared to carbon fibers alone.
- Modifying the surface of the C/C composite with CNT after functionalization does not increase the electrical conductivity of the composite but significantly improves electrochemical parameters - the materials are more active electrochemically;
- The presence of functional groups on the surface of the CNT significantly improves the CIC value, which is an important parameter from the point of view of nerve cell stimulation;
- No cytotoxic effect was observed in any of the analyzed samples both before and after surface modification;
- The preliminary results of the cell viability test on the C/C composite samples are at the level of the values obtained for the Pt wire and are higher than for the CF sample.

This research was funded by the National Science Centre (UMO-2020/39/B/ST5/02126)
(2021-2025)