INTRODUCTION

Novel biocompatible materials (Ratner, 2011; Williams, 2008) are important in different medical applications, for example implantology (Barr, Hill, & Bayat, 2017; Pałka & Pokrowiecki, 2018), wound healing (Ishihara et al., 2002; Schneider, Wang, Kaplan, Garlick, & Egles, 2009; Shanmugapriya & Kang, 2019), drug delivery (Langer & Peppas, 2003; Mura, Nicolas, & Couvreur, 2013; Peer et al., 2007) and many others (Guimard, Gomez, & Schmidt, 2007; Katz & Willner, 2003). Nowadays, one of the mostly developed field of research is nanomedicine (Moghimi, Hunter, & Murray, 2005; Shi, Kantoff, Wooster, & Farokhzad, 2017; Wagner, Dullaart, Bock, & Zweck, 2006), based on tremendous diversity of nanomaterials, especially metallic (Chithrani, Ghazani, & Chan, 2006; Kim et al., 2007; Kuppusamy, Yusoff, Maniam, & Govindan, 2016), oxide (Kasemets, Ivask, Dubourguier, & Kahr, 2009; Kaviyarasu, Geetha, & Kanimozi, 2017; Pankhurst, Connolly, Jones, & Dobson, 2003), polymer (You et al., 2007; Zalusky, Olayo-Valles, Wolf, & Hillmyer, 2002), and of course carbon nanomaterials (Nurunnabi et al., 2015; Schrand, Hens, & Shenderova, 2009; Yang, Thordarson, Gooding, Ringer, & Braet, 2007; Zhang, Grünner, & Zhao, 2013). Besides their composition also the size, architecture and surface properties have a huge impact on the interactions and activity in contact with a human body (Nel et al., 2009). After years of studies, scientists are still looking for safe, stable materials which can be multi-effective after injection into human body (Williams, 2019). The complexity of biomaterials is needed to obtain the best biocompatibility simultaneously with anti-inflammatory (Anderson, Rodriguez, & Chang, 2008) and anti-bacterial (Moritz & Geszke-Moritz, 2013) (but also much more specific) properties related to the targeted application.

One of the promising approach is the preparation of various layered carbon materials, for example containing different carbon
fibres, carbon nanotubes, nanohorns, nanonions, graphene oxide and others. This is because a majority of them has great biocompatibility (d'Amora et al., 2016; He et al., 2018; Lettieri, Camisasca, & d'Amora, 2017). Simultaneously, they have various morphologies and very specific properties, which can be used to modify a material in a proper way. Especially, spherical carbon nanomaterials are very promising in the field of surfaces nanocoating. They are highly biocompatible (d'Amora et al., 2016; Lettieri et al., 2017; Zhu et al., 2012) and have a structure allowing easy doping and modification. Because of conductivity and the ability to form rough coating, they can stimulate cells and tissue to regeneration (Liao et al., 2006).

Also use of the carbon fibres in biomedical aspects is quite common (Li et al., 2007; Wang, Ma, & Cheng, 2003). They have superior mechanical properties, are bio-inert and chemical stable, noncorrosive, resistant for degradation (Saito et al., 2011; Simon, Prosen, & Duffy, 1967), so it is obvious that they have been used as a base for implants or scaffolds for cell growth (Hutmacher, 2000). However, their surface area is usually low (Huang et al., 2012). Implants and other biomaterials not only need to match the tissue in macro and micro scale, but also in the nanoscale—via some specific interactions with cells (Liao et al., 2006). This is why such an important problem is to find a proper cover—so-called nanocoating. Together, carbon fibres and spherical carbon nanomaterials can be used for preparation of a layered material accelerating tissue regeneration and cell growth. They can also be easily modified and customized with dedication to some very specific applications.

The idea of the presented study is to prepare the material based on a carbon fibre (CF) cloth covered with spherical carbon nanomaterials (nanodiamonds—ND and carbon nanonions—CNO) by the electrophoretic deposition (EPD). Moreover, we use exactly the same conditions of the EPD process for all studied carbon nanomaterials, and the EPD takes place exactly on the same surface of a CF. This makes it possible to compare the properties of obtained surfaces. They are characterized and tested for stability in aqueous environment and for a biocompatibility.

## 2 | EXPERIMENTAL

Deposition processes were carried out on the surface of a CF purchased from the HP-textiles GmbH (art. no. HP-T193C) purified by a heating at 500°C in N₂ (95%) for 3 hr, between previously prepared carbon papers for the polymer coating sorption and removal of the other impurities. In the experiments, four different spherical carbon nanomaterials were used: commercial ND (<10 nm; 97% purity; Sigma Aldrich), CNO obtained by an annealing of ND, prepared as described in previous research (Plonska-Brzezinska, Dubis, Lapinski, Villalta-Cerdas, & Echegoyen, 2011) (CNO-ox), and CNO obtained in our laboratory by underwater (in distilled water) arc discharge (Iwasaka, Kanatake, Ohshiro, Suehiro, & Hara, 2006) of spectrally clear carbon rods (Carbo Graf) at 50 and 70 A (CNO-50, CNO-70) based on the Sano method (Sano et al., 2002).

### 2.1 | Electrophoretic deposition

Suspensions of carbon nanomaterials 0.1 mg/ml (200 ml) in deionized water were prepared by a triplicate sonication for 5 min 60 W with 2 min breaks to prevent overheating. After 10 min of possible sedimentation, suspensions were used for the deposition.

The EPD processes (at the room temperature) were carried out under 100 V/cm for 3 min with the distance between electrodes equal to 10 mm. Applied potential was used as a maximum value to obtain uniform coating with possibly high deposition efficiency. Chosen time is known as a limit value of the deposition speed, after 3 min the process drastically slows down, and this phenomenon does not depend on the applied voltage (Wang, Leu, & Hon, 2004). The electrodes were carbon fibres and nickel, respectively. Depending on the ζ-potential of the nanomaterial the EPD was carried out on the positive or negative electrode. After deposition, the samples were washed out with ethanol (96%) and dried at 70°C in air for 5 min.

Just before the water contact angle (WCA) measurements, the samples were desorbed at 130°C in air for 20 hr and slowly cooled down in a glass container to avoid the adsorption of impurities.

The efficiency of the electrophoretic deposition was estimated after the sonication of obtained samples in 10 ml of deionized water for 10 min with 10 s pulsations in 60 W. Next, the calibration curves were prepared and the amount of the removed nanomaterial was measured by the UV-vis spectrometry (the wavelength equal to 225 nm).

### 2.2 | Characterization methods

Initial nanomaterials used for the deposition were characterized by a transmission electron microscope (Tecnai F20 X-Twin; FEI). The BET surface area was measured by low-temperature (~196°C) N₂ adsorption-desorption (Gemini, Micromeritics). Raman spectrum was measured in a solid phase using a 785 nm laser with power 2 mW (Senterra, Bruker Optik). XRD patterns were recorded for powders (Philips X′Pert with X′Celerator Scientific detector), and thermal stability was based on the thermogravimetric analysis (Netzsch, Jupiter STA 449 F5) in N₂ at the heating rate 10°C/min. DLS and ζ-potential (25°C, Particulate Systems, NanoPlus HD, Micromeritics) of suspensions were measured using solutions having a concentration of 0.1 mg/ml (volume 20 ml) after 5 min sonication (60 W; BANDELIN, Sonopuls HD 4100).

Covered carbon fibres were characterized by a scanning electron microscope (Quanta 3D FEG). Roughness factor was calculated (program NanoScope Analysis) based on the atomic force microscope analysis (MultiMode with scanner type E, tapping mode). The WCA was measured (at 25°C) three times for each sample using homemade goniometer having a fixed-focus lens (as reported previously, Terzyk, Bryk, & Korczewiński, 2019) with a new camera Grasshopper3 GS3-U3-3254C-C, 3.2 Mpx. To define the changes in surface potential of materials, flat surface
measurements of ζ-potential were conducted, using the flat surface cell (25°C; Micromeritics) and the method described by Corbett, McNeil-Watson, Jack, and Howarth (2012). To do this, we used the sample monitor solution with tracer particles produced by Otsuka Electronics Co.

### 2.3 In vitro tests

The idea of the possible future application of prepared composites may be using them as an artificial skin to cover damaged tissue.

**Table 1** The comparison of selected properties of CNO-50, CNO-70, ND and CNO-ox, both DLS and ζ-potential analysis were carried out in water with concentration 0.1 mg/ml.

|          | Size | BET     | ζ-potential |
|----------|------|---------|-------------|
|          | TEM  | DLS     | [m²/g]      | [mV] |
| CNO-50   | 41 ± 11 | 230 ± 40 | -29.87 ± 0.24 |
| CNO-70   | 17.2 ± 7.3 | 218 ± 29 | -32.8 ± 1.3 |
| ND       | 6.4 ± 1.4  | 1,520 ± 630 | -11.87 ± 0.15 |
| CNO-ox   | 6.4 ± 1.3  | 520 ± 51  | 5.52 ± 0.97 |

**Figure 1** TEM images of initial CNO-50 (Hetmann et al., 2018) (a, b), CNO-70 (c, d), ND (e, f), CNO-ox (g, h).

**Figure 2** Raman spectra (upper) and XRD patterns (below) obtained for CNO-70, CNO-50, ND, CNO-ox.
and probably accelerate the regeneration, so HDF-n cell line were used as a model for human skin to receive some basic information about their response to the used materials. The in vitro tests of covered samples were performed by an extract method based on ISO 10993-5 (Han et al., 2019). Extracts were derived from soaking the samples with DMEM cell culture medium for 24 hr at 37°C (Han et al., 2019). HDF-n (neonatal Human Dermal Fibroblasts, ScienCell) cell line was cultured according to the manufacturer’s protocol under sterile conditions at 37°C in 4.9% CO₂. After thawing, cells were cultured until they reach sub-confluency state. Then, the cells were detached using 0.25% trypsin solution, and 1 × 10⁵ cells/well were seeded in 12-well plates.

2.4 | MTT assay

The cell viability and proliferative potential based on their metabolic activity was determined after 24 hr with direct and indirect MTT assay. In direct test, materials after previous conditioning for 24 hr in water solution were placed in the plate wells (as a cell substrate), and cellular response was caused by cell contact with the samples. In indirect test, extracts were added to adherent cells and cellular responses were caused by secreted compounds of materials. After 24 hr exposition, cells were incubated with MTT reagent (0.5 mg/ml) for 30 min at 37°C. Obtained formazan crystals were dissolved in 1 ml of DMSO. The results were read spectrophotometrically at a wavelength of 570 nm. Each experiment was carried out in duplicate.

2.5 | Neutral Red Uptake (NRU) assay

Neutral Red Uptake assay bases on the uptake of the neutral red dye by the viable cells and its accumulation in the lysosomes (Ivask et al., 2015). In the test, the extract was added to adherent cells and cellular response was studied after 24 hr exposition. Then, the extracts were removed and fresh medium with 0.033% neutral red dye added and incubated for 45 min at 37°C. After that, dye was removed and cells were washed with warm PBS buffer.

| TABLE 2 | Roughness factors, flat surface ζ-potential analysis and water contact angle for obtained surfaces: CF, CF-CNO-70, CF-CNO-50, CF-ND, CF-CNO-ox |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
|         | CF       | CF-CNO-70 | CF-CNO-50 | CF-ND    | CF-CNO-ox |
| Rq [nm] | 46.4     | 69.7      | 83.0       | 135.0    | 72.5       |
| ζ-potential [mV] | 0.32 ± 0.59 | -0.82 ± 0.55 | 0.21 ± 0.37 | -0.84 ± 0.6 | -0.21 ± 2.08 |
| WCA [°] | 71.5 ± 5.9 | 130.3 ± 12.2 | 148.1 ± 13.6 | 139.5 ± 12.9 | 152.6 ± 4.6 |
| Deposition efficiency [mg/g] | –       | 0.29 ± 0.12 | 0.0900 ± 0.0014 | 1.808 ± 0.087 | 1.09 ± 0.41 |
incorporated neural red dye was released by adding the 1% acetic acid in 50% ethanol. The results were read spectrophotometrically at a wavelength of 540 nm. Each experiment was carried out in three replicates.

2.6 | In vitro scratch assay

The scratch assay allows the measurement of cell migration and mimics the wound healing process (Liang, Park, & Guan, 2007). Procedure was based on the Nature Protocols. After cells were cultured, the x-shaped scratch in a cell monolayer was created. From then, cells were cultured in the extract, and the distance between cells in a scratch was measured after 24 and 48 hr in the same place. Each experiment was carried out in duplicate.

FIGURE 5 3D height images of obtained surfaces: CF (a), CF-CNO-70 (b), CF-CNO-50 (c), CF-ND (d), CF-CNO-ox (e), obtained during AFM tapping mode analysis

3 | EXPERIMENTAL RESULTS

3.1 | The comparison of nanomaterials

Figure 1 shows the TEM images of the initial spherical carbon nanomaterials. They have different size and shape, and the commercially available nanodiamonds and CNO-ox obtained by the annealing of ND are smaller with the largest surface areas (Table 1). CNO-50 are the largest, but still almost spherical (Figure 1a,b). It is also visible that it is a layered material with negligibly small amount of carbon nanotubes. As it is shown in Figure 1c,d, CNO-70 are small, just twice larger than ND, but noticeably oval and elongated. Also, small amount of amorphous carbon is visible on the surface of the CNO-50 and CNO-70. This is a residue after the synthesis and because of the very small quantity, and its influence on the materials properties
can be ignored. Both nanoonion samples obtained by the underwater arc discharge have low surface area values (Table 1). As it is seen, all studied nanomaterials create aggregates, what also was proved by the results of the DLS analysis (Table 1). Also ζ-potential analysis shows the difference between CNO obtained by an arc discharge method and nanodiamond-based materials. The lower value of ζ potential, near −30 mV means that a colloid is more stable and the deposition will be probably less effective, than from the suspension with ζ-potential closer to 0 mV (see below) (Besra & Liu, 2007). This difference in potential for different nanoonions is a consequence of the synthesis methods used for their preparation. Samples obtained by underwater arc discharge have relatively low ζ-potential, what leads to the conclusion, that during synthesis they can be oxidised (because of the aqueous environment). However, it is also possible that adsorption of hydroxyl ions leads to this charge, because it is well known that this effect occurs for strongly hydrophobic surfaces. On the other hand, CNO-ox are prepared by annealing of ND in helium, so before the oxidation, all of the functional groups have been desorbed. The oxidation was performed under the mild conditions, so the amount of the functional groups is not large enough to dominate the average ζ-potential of the nanomaterial. Moreover, our previous studied showed that this oxygen is mainly bonded to carbon as surface carboxyls (Beattie & Djerdjiev, 2004; Tian & Shen, 2009), leading to positive charge of carbon surface. Presented on

**Figure 6** SEM images obtained for CF (1, a, b), CF-CNO-70 (2, c, d), CF-CNO-50 (3, e, f), CF-CNO-ox (4, g, h), CF-ND (5, i, j)
Figure 2 XRD patterns, Raman spectra, as well as the results of thermal stability measurements (Figure 3), show significant differences between samples. Raman spectrum for nanodiamonds (Figure 2) has weakly visible signals, due to hygroscopic nature of this material and it corresponds well with previous results obtained for this material by Gubarevich et al. (2003) Raman spectra of carbon nanomaterials have two main bands: the G-band connected with graphitization (~1,570 cm$^{-1}$), the D-band (~1,340 cm$^{-1}$) correlated with defects in crystalline structure and presents of amorphous carbon (Larouche & Stansfield, 2010). Additionally, 2D-band (~2,700 cm$^{-1}$) is linked to stacking order of graphite layers (ABAB) (Codorniu Pujals & Arias de Fuentes, 2015; Ferrari, 2007). The ratio of the intensities of D and G peaks estimate the degree of perfection of graphene planes. CNO-50 and CNO-70 have great graphitic structure ($I_D/I_G$ CNO-50 = 0.45, $I_D/I_G$ CNO-70 = 0.38), on the other hand D-band of CNO-ox is much more intense($I_D/I_G$ CNO-ox = 1.18). Firstly, the oxidation of nanomaterial cause defects in its structure. Secondly, materials were obtained from different methods. CNO obtained by annealing has a lower ID/IG ratio than this obtained by arc discharge(Alessandro et al., 2018; Borghoain, Yang, Selegue, & Kim, 2014; Mykhailiv, Zubyk, & Brzezinski, 2017; Rosenkranz et al., 2018). All of the differences (the size of a single particle, the structure and the stability) have significant influence on the results of subsequent analysis.

3.2 | Characterization of surfaces

After deposition, surface $\zeta$-potential of the samples is quite similar for all the materials and is dominated by potential of pure carbon fibres (Table 2). However, taking into account the measurement error one can conclude that for two samples, namely CF-CNO-70 and CF-ND, the surface $\zeta$-potential is negative. Generally, the surface $\zeta$-potential measurement is not so sensitive for the changes of the surface nature as the WCA measurement is. Further comparative studies are necessary, and the results will be reported. The WCA value for the initial CF shows that this surface is hydrophilic, and the deposition of spherical carbon nanoobjects leads to the switch of wetting properties (i.e., the surface becomes hydrophobic).

They depend on the roughness factor ($R_q$) of a surface and the $\zeta$-potential of the initial nanomaterials, as it is shown in Figure 4. The rise in $R_q$ for the hydrophobic surface, according to the Cassie-Baxter effect (Cassie & Baxter, 1944) leads to the rise in the WCA value. It is important that the thickness of a layer is relatively small (see below); however, the differences between the WCA values for obtained surfaces are significant.

However, the more negative is the $\zeta$-potential of the initial material, the better wettability is (Figure 4). It means that oxygen groups are present on a substrate surface, and by the interaction with a droplet, they lead to the rise in the work of adhesion and the decrease in the WCA value. In contrast, for the CNO-ox sample (having the largest and positive $\zeta$-potential value) the contact angle is the largest.

Additional information on the structures of surfaces is obtained from the data collected on Figure 5, showing the profiles obtained by the AFM method. All spherical carbon nanoobjects are deposited as aggregates (see also Figure 6). Taking into account the results of the DLS measurements for the initial nanomaterials (Table 1), one can conclude that the process is dominated by the deposition of aggregates present in the initial solution (i.e., generally the $R_q$ values increase with the rise in the DLS diameter—see Tables 1 and 2). This is why for CNO-50 and CNO-70 coverings are not uniform, less homogenous and thinner compared to samples with deposited ND and CNO-ox. However, the latter sample is quite smooth, and the deposited material creates dense and very homogenous covering.

We would like to point out that all conditions were chosen as the best for the electrophoretic deposition process to obtain the most homogeneous covering. Presented results show that it is impossible to receive the same effect using different nanomaterials. It also has the reflection in the deposition efficiency presented in Table 2. To explain the details of the EPD process, further studies are necessary (i.e., EPD from different solvents) and the results will be reported.
3.3 Cytotoxicity tests

The results of cytotoxicity tests for obtained surfaces are presented on Figure 7. As it is shown in this image, all materials are more biocompatible than pure carbon fibres. It is clear that in the indirect tests the most cytotoxic surfaces are the least stable. Thus, pure CFs do not release any harmful materials, but at the same time the samples covered with CNO-ox and ND (the thickest covering) are releasing bigger amount of weakly connected nanomaterials than CNO-50 and CNO-70. As a result, released nanomaterials can interact with cells, or even coming through the cell membrane and cause decrease in cells viability. Similar situation is visible in the NRU test results; however, here the sample with ND seems to be less toxic than other materials. It can be a result of high viability of cells visible for the NRU test, but their metabolic activity is lower (what is measured in indirect MTT assay).

In the direct MTT test, it is seen that covering in significant way increases the metabolic activity of cells. Comparing both indirect and direct toxicity tests results, it is clear that nanomaterials obtained by the underwater arc discharge create more stable and less toxic surfaces than the materials based on nanodiamonds; however, conditioning of the samples before the use can remove the weakly bonded excess from the surface of CFs and then the covering also can be more biocompatible than the CF. The scratch assay results show that extracts from all covered samples in significant way increase the cells migration comparing to the pure carbon fibres. Obtained results correspond with the direct MTT test and give the possibility to use obtained materials for future application in biomedicine (Figures 8 and 9).

4 CONCLUSIONS

Comparing all samples obtained by the EPD of the spherical carbon nanomaterials on the surface of purified CFs, it is clear that there are significant differences in morphology, structure and properties between nanomaterials obtained using different preparation methods (underwater arc discharge and nanodiamonds-based). All dissimilarities influence on the deposition process and have huge impact on the homogeneity and stability of the obtained coatings. It also leads to the differences in the potential use in plausible biomedical applications. After cytotoxicity tests more promising seem to be layered materials of CFs with CNO-50 and especially CNO-70, as more stable and biocompatible materials.

However, the idea of preparing two-layered carbon-carbon materials from CFs and spherical nanomaterials leads to increasing biocompatibility, comparing to pure CFs. They retain strength and
flexibility of CNOs with all the advantages of nanoscale materials where the use of carbon nanomaterials coatings offers additional opportunity to deliver drugs or other active substances to the body. This also offers possibilities for future studies and experiments towards searching advanced drug delivery systems, and also wound healing dressings or soft tissue implants.

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