Magdalena Ziąbka[1,*], Michał Dziadek[2]

Surface Properties of Polymeric Composites with Silver Nanoparticles

DOI: 10.5604/01.3001.0012.5169

[1] **AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Ceramics and Refractories,**
Kraków, Poland
e-mail: ziabka@agh.edu.pl

[2] **AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Glass Technology and Amorphous Coatings,**
Kraków, Poland

Abstract
The aim of this study was to investigate the surface properties of polymeric composites and the osteoblastic cell behaviour set in direct contact with the biomaterials tested. The surface properties were evaluated before and after 6-month incubation in an in vitro environment. The composite materials were prepared by means of extrusion and injection moulding. Three commercially available thermoplastic polymers (ABS (poly)acrylonitrile butadiene styrene) were used as composite matrices. Antibacterial silver nanoparticles (AgNPs) were added as a modifying phase. Surface properties of the materials tested, such as: wettability, roughness and microstructure, were determined. Furthermore the morphology of Saos-2 human osteoblastic cells in direct contact with the composite materials was assessed after the 7-day culture. The addition of silver nanoparticles caused minor changes in the wettability and roughness values. As light modification, the silver nanoparticles influenced the microstructure. The osteoblasts displayed the proper morphology and they evenly settled on the surface of the pure polymer and composite materials, which indicated the material cytocompatibility.

Key words: *surface properties, nanocomposites, silver nanoparticles, biomedical polymers.*

Introduction

The impact of biomaterials on the biological environment is a complex phenomenon in dependence on their physicochemical properties. Well-selected properties of implantable materials are a key factor to ensure successful osteointegration. The crucial properties of biomaterial include the surface topography, roughness, thread design, implant-abutment connection, surface chemistry, wettability and surface modifications.

The qualities biomaterials are endowed with should match the specificity of the application site and the role the implant is supposed to play. That is why it is a common necessity to modify the biomaterial surface to make it more advantageous in direct contact with biological structures.

The beneficial surface properties of implantable polymer composites are a crucial requirement concerning their functionality. The polymer interacts with the biological environment in the range up to 5 Å, which means that the outer layer of the polymer plays a much more vital role than the core [1].The implant not only has to function properly but also withstand degradation within the progress of time. Therefore it is essential to retain the key properties of the biomaterial while modifying its surface to facilitate bio-interaction. The aim of all the surface modifications is to increase bone-implant osteointegration [2]. It is also important to select the proper material, as it is known that certain types of bioactive ceramics, e.g. hydroxyapatite and bioglass, may accelerate osteointegration after implant insertion [3].

Nowadays it is necessary to develop novel biomaterials due to the growing antibiotics-resistance of bacteria. One of the highly-regarded solutions is applying nanoparticles of precious metals, as they display potent antibacterial and antifungal properties. Among them, the most popular choice is silver [4]. Silver compounds and ions have been extensively used for both hygienic and healing purposes thanks to the broad spectrum of antimicrobial activity [5, 6]. Silver shows a multilevel antibacterial effect as it blocks respiratory enzyme pathways, and alters the microbial DNA and cell wall [7]. Silver has also proven to be effective against multidrug-resistant organisms [8, 9], whilst maintaining a low systemic toxicity [10]. Several clinical studies have confirmed silver-modified composites to be safe [11, 12] and concerns about their cytotoxicity on fibroblasts and keratinocytes have not been confirmed [13-15]. That is why, silver is commonly used in many fields of medicine, e.g. as an ingredient of bandages, patches and ulceration treatments [16, 17].

The advantageous pro-health properties of silver were discovered a long time ago. In ancient Greece plates and cups were covered in silver to prevent the spread of diseases [18]. Using silver crockery was popular in the Middle Ages as well. In 1884 the first treatment involving silver was noted, which was the silver nitrate applied to cure the bacterial eye inflammation of babies. Also syphilis and tonsilitis were treated with silver until the antibiotics era came [19].

The mechanism of the silver phenomenon has not been entirely explained so far. Silver interacts with the thiol groups of bacteria enzymes [20], which results in the enzyme and protein expression [21]. Another explanation of silver bactericidal and fungicidal activity is its ability to break the cell membrane, cause protein denaturation and oxygen radicals formation, and exhibit DNA or RNA replication [22].

The antibacterial properties of silver are also dependant on the size and shape of its particles. Nanoparticles act multi-directionally thanks to their tiny size and large area of the inter phase with the bacteria cell membrane [23].

For a long time highly-reactive Ag^+ ions were considered to be the only toxic form of silver. However, recent studies have shown that silver nanoparticles themselves have a negative impact on cells, since they probably interfere with cell membrane activity. They diffuse across the cell membrane and release reactive Ag^+ ions, which negatively affects the sensitive organelles inside.

So far silver nanoparticles have displayed a negative impact on living organisms in a number of ways. The work of Arora et al. [24] proved that silver aggregates accumulated in mitochondria and cyto-

plasm, which led to disturbances in the antioxidant enzyme system and increased the production of reactive oxygen forms that are toxic to cells. The studies also revealed negative changes in cell morphology depending on the silver nanoparticle concentration. Poon et al. [25] proved that silver applied in burn treatments entirely inhibited the metabolism and proliferation of healing cells. It was concluded that Ag^+ ions released from silver are not only selectively targeted at the bacteria but they affect all the cells, including those involved in the healing process. The observations revealed that nanosilver causes cell necrosis or apoptosis depending on the particle concentration. The silver present in mitochondria leads to oxidative stress and obstructs the antioxidative defence mechanism, thus the production of reactive oxygen forms is increased. Consequently the lipid peroxidation, lipoprotein modification and the destruction of proteins and nucleic acids occur, leading to inflammatory and degenerative processes. The highly negative result might be the apoptosis and necrosis of mitochondria [26]. Yet another consequence of oxidative stress is metabolic disorder and damaging cell membrane integrity. Genotoxicity is the most disadvantageous effect of silver nanoparticles activity.

Taking into account all the aspects, it is crucial to modify biomaterials so as to achieve the most beneficial biological properties. It is essential to maintain a balance between bactericidal and cytotoxic activity. The amount of nanosilver has to eliminate the bacteria, yet it should not inhibit cell proliferation.

The present work focuses on the correlation between the surface properties of polymer composites and the amount of modifying phase applied.

Materials and methods

Materials were prepared using a vertical injection moulding machine with three heating zones (Multiplas). Three commercially available polymers (ABS (poly)acrylonitrile butadiene styrene, INEOS Styrolution) and composite materials modified with 0.5% and 1% wt. silver nanoparticles AgNPs (NanoAmor company) were shaped as discs measuring 10 mm in diameter. The names of the polymer products and their physico-chemical properties are presented in ***Table 1***.

Table 1. *Physico-chemical properties of polymers.*

	Young's modulus, MPa	Tensile strength, MPa	Melting point, °C
1. Novodur HD 15	2300	102	230-260
2. Novodur HD M203FC	2400	110	230-260
3. Novodur HD M205FC	2550	120	230-260

Table 2. *Type and nomenclature of samples.*

Sample description	Sample name
Novodur HD M203FC	NHDM203FC
Novodur HD M203FC with 0.5% volume fraction of AgNPs	NHDM203FC_0.5Ag
Novodur HD M203FC with 1% volume fraction of AgNPs	NHDM203FC_1Ag
Novodur HD M205FC	NHDM205FC
Novodur HD M205FC with 0.5% volume fraction of AgNPs	NHDM205FC_0.5Ag
Novodur HD M205FC with 1% volume fraction of AgNPs	NHDM205FC_1Ag
Novodur HD 15	NHD15
Novodur HD 15 with 0,5% volume fraction of AgNPs	NHD15_0.5Ag
Novodur HD 15 with 1% volume fraction of AgNPs	NHD15_1Ag

The procedure of obtaining samples consisted of a few steps. First the granulate was prepared and dried in a laboratory dryer at 80 °C for 6 hours. Next it was melted and homogenised with nanosilver particles in a plasticising chamber. Subsequently the material was injected into a steel moulding form, cooled and ground [27]. The process of homogenisation was carried out in a dual cycle, where the compositions were melted twice. The injection parameters were selected according to the manufacturers' recommendations and tailored to each composition. A scheme of the samples' production is presented in ***Figure 1***.

The samples obtained in the thermoplastic processing are summarised in ***Table 2***.

The polymer and composite samples were examined to assess their surface properties. Tests were carried out on the initial samples (immediately after manufacturing) and on those incubated in deionised water for 6 months in a laboratory dryer at a temperature 36 °C ± 1 °C. Tests were run to evaluate the effect of the modifying phase on the surface properties of the polymers in conditions imitating the biological environment within the progress of time.

Wettability examinations were performed to assess the hydrophilic/hydrophobic character of the surface. Surface roughness was established to describe the topography of the polymers and nanocomposites as well as the nanosilver

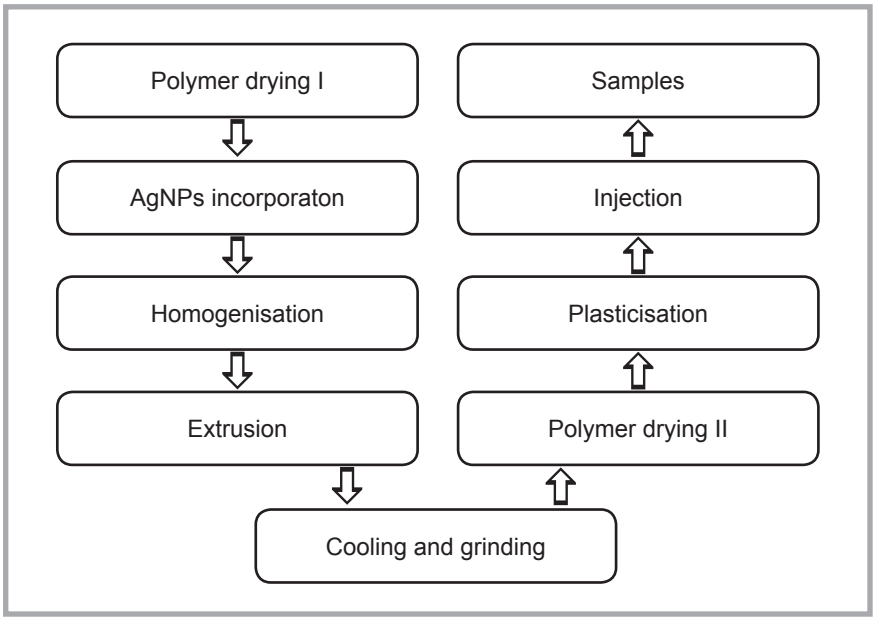

Figure 1. *Scheme of samples' preparation.*

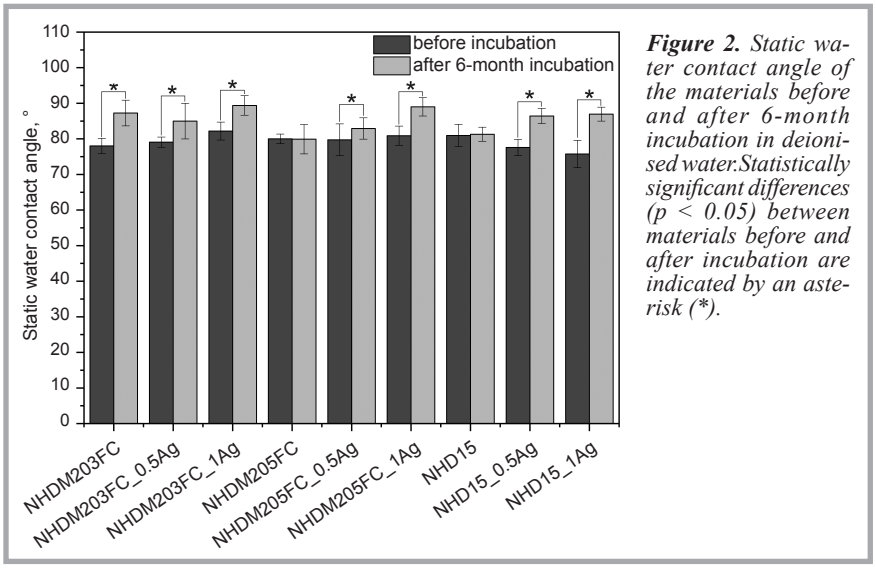

Figure 2. *Static water contact angle of the materials before and after 6-month incubation in deionised water.Statistically significant differences ($p < 0.05$) between materials before and after incubation are indicated by an asterisk (*).*

distribution on the nanocomposite surface. The surface microstructure and cross-sections of the polymers and nanocomposites were evaluated using a scanning electron microscope.

The surface roughness parameters were evaluated using a contact profilometer – HOMMEL-ETAMIC T1000 wave (Jenoptik AG, Germany). The arithmetical mean roughness (Ra) and ten point mean roughness depth (Rz) of the surfaces were determined.

The surface wettability was evaluated by means of static water contact angle measurements. The contact angle was determined by the sessile drop method with an automatic drop shape analysis system – DSA 10 Mk2 (Kruss, Germany). UHQ-water droplets of 0.25 µl were applied on each pure and dry sample. Measurements were carried out in constant conditions of temperature and humidity.

The surface roughness parameters and apparent contact angle were calculated as an average of 10 measurements and were expressed as a mean ± standard deviation (SD).

A Nova Nano SEM FEG-200 scanning electron microscope (FEI, Netherlands) was used to perform a detailed examination of the samples' microstructure. The measurements and observations were conducted in low vacuum conditions, using a secondary electron detector (SE) with an accelerated voltage of 10-18 kV. Microstructure observations were conducted on the surface and cross-sections of the samples before and after incubation. All the samples were coated with a carbon layer.

The results were analysed using one-way analysis of variance (ANOVA) with Duncan post hoc tests, which were performed with Statistica 13.1 (Dell Inc., USA) software. The results were considered statistically significant when $p < 0.05$.

In vitro biological evaluation of the materials was carried out using a Saos-2 cell line (human osteosarcoma, ATCC, USA) from 3 passages. In order to obtain a cell suspension, the culture was washed twice with phosphate buffered saline – PBS, followed by a 5% trypsin solution with EDTA (HyClone, USA). After washing and centrifugation, the cells were suspended in a fresh medium. Next 1 ml of the resulting cell suspension, at a density of 10^4 cells/ml, was added to the wells of 48-well culture plates (Nunc, Denmark) containing the sterile discs of the materials. Cell culture of the discs in direct contact with the materials tested was carried out for 7 days.

FEI Nova NanoSEM 200 scanning electron microscope was used to perform a detailed morphology examination of the cells adhered to the materials investigated. Observations were conducted in high vacuum conditions using a back scatter electron detector (BSE) at an accelerated voltage of 10-18 kV. After 7 days of the cell culture, the materials were rinsed with PBS, and then the cells were fixed with 3% glutaraldehyde solution in a sodium cacodylate buffer,

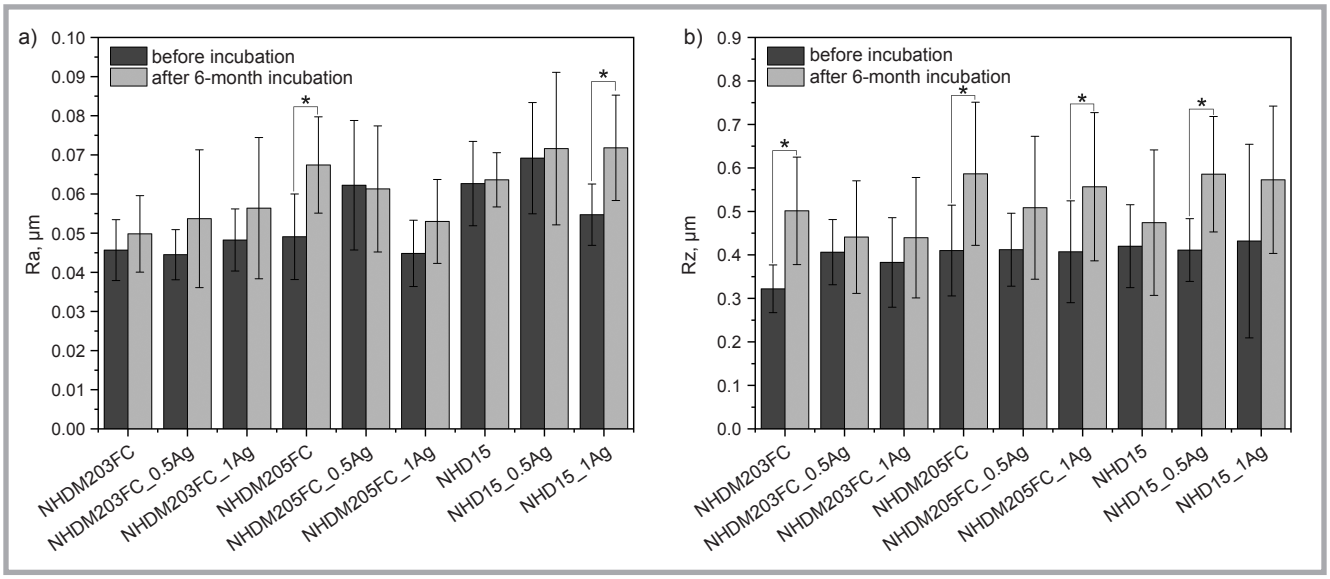

Figure 3. *Average roughness (Ra) and ten point mean roughness depth (Rz) of the materials before and after 6-month incubation in deionised water. Statistically significant differences ($p < 0.05$) between materials before and after incubation are indicated by an asterisk (*).*

pH 7.4 (POCh, Poland) for 30 minutes. Subsequently the cells were dehydrated in a graded series of ethanol solutions (70%, 80%, 90%, 96% and 100%) and dried in air. The cell morphologies were evaluated after the carbon coating.

Results and discussion

The surface properties were evaluated after testing the samples (***Figure 2***). For all the materials the water contact angle values did not exceed 85° before the 6-month incubation in deionised water, which proved their hydrophilic character. Adding the nanosilver modifier did not have a significant influence on the surface wettability. The samples incubated in deionised water for 6 months revealed a slight increase in the wetting angle, yet none of the materials displayed a value of more than 90°.

The basic roughness parameters (Ra, Rz) were obtained (***Figure 3***). The irregularity of the polymer and composite surfaces was not high. The profilometry tests proved that all the materials were characterised by an arithmetic average of the roughness profile (Ra) below 0.09 µm, which indicated low surface roughness. The Rz parameter value – i.e. a ten point mean roughness depth – was low as well, whose range of 0.32-0.43 µm proved that only small areas were endowed with higher roughness. These were the sites where nanosilver and its agglomerates were located close to the surface. Having been incubated in deionised water, the samples revealed slightly higher roughness values. Probably such an increase resulted from uncovering the nanoparticles and silver agglomerates on the sample surface. Our previous works [28] proved that nanoparticles may have been partially washed away, increasing the material roughness.

Composites enriched with bioactive nanoadditives may show increasing roughness, which promotes cell adhesion and proliferation. It is well known that cells better expose themselves to nanoscale surface roughness than flat and smooth surfaces [29].Therefore it is very important to create nanostructured surfaces that are more biomimetic comparing to standard flat culture surfaces [30]. Bourkoula et al. [31] proved that different cells may behave differently on rough surfaces, the cause of which can be found in the double role of elasticity and size, which may be found in cell viability on rough surfaces:

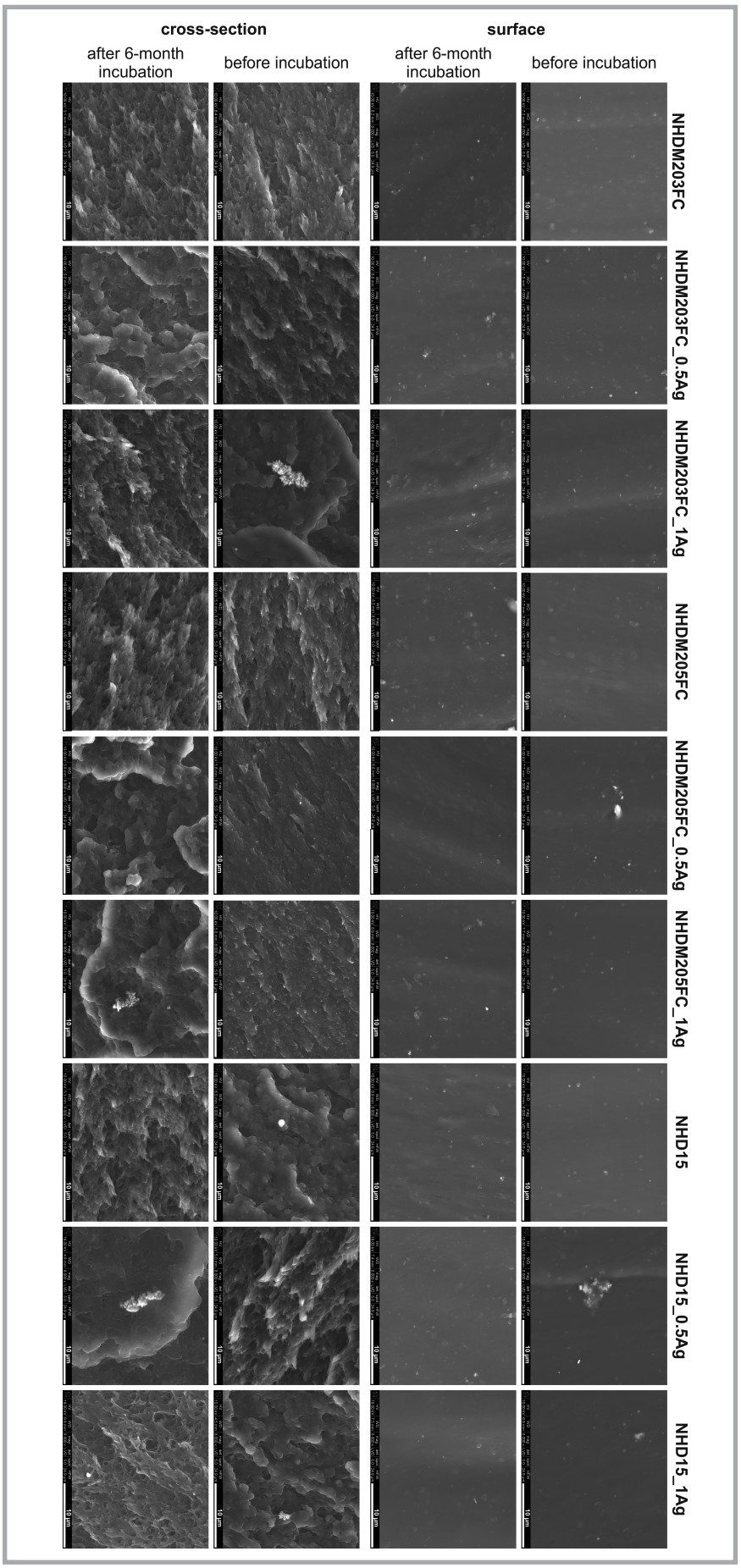

Figure 4. *SEM images of the surface and cross-section of the materials before and after 6-month incubation in deionised water. Magnification 8 000x.*

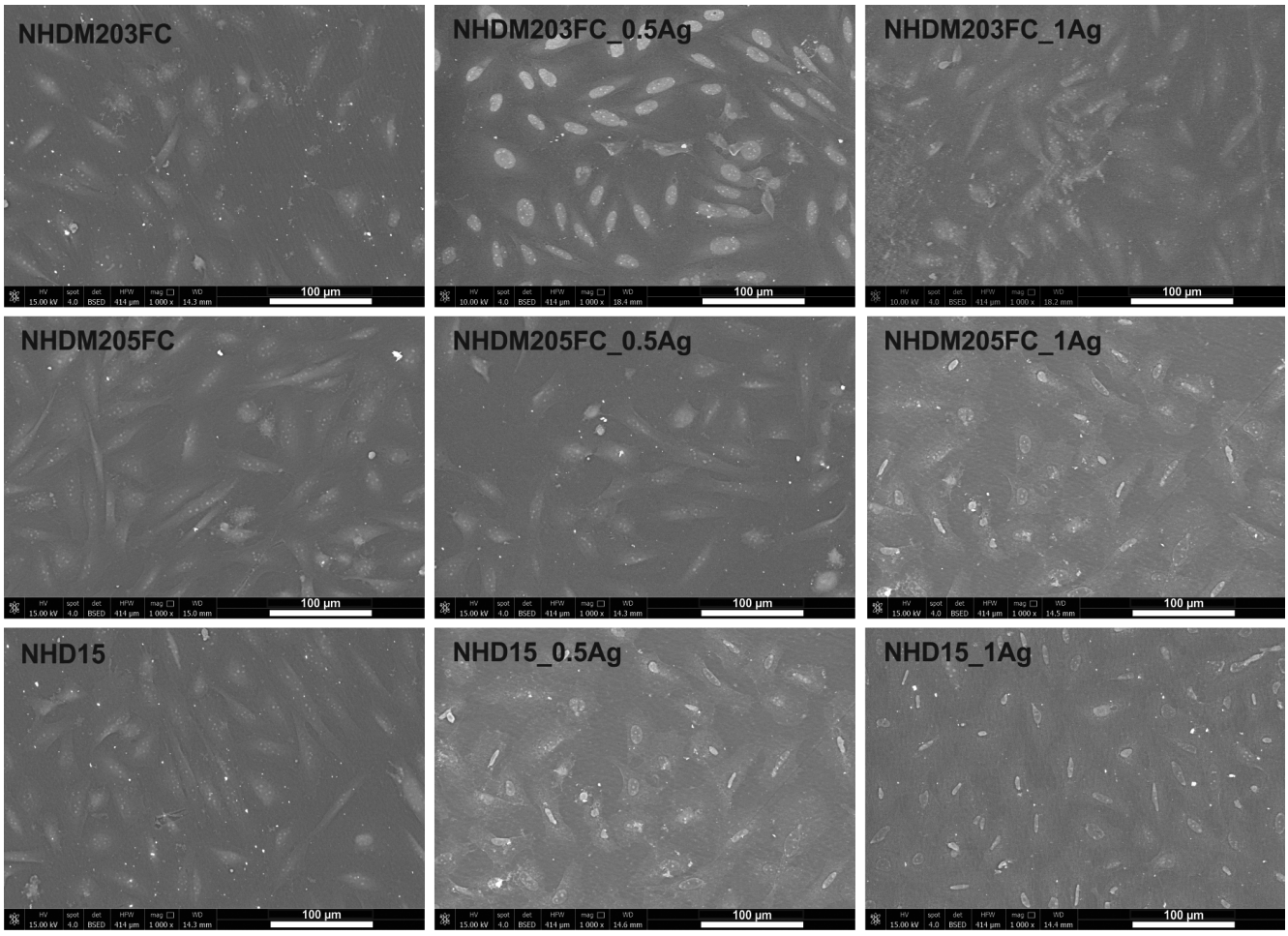

Figure 5. *SEM images of Saos-2 cells in direct contact with the materials tested after 7-day culture. Magnification 1 000x.*

on the one hand they enhance adhesion areas on surface protrusions, thereby increasing the capturing strength, while on the other hand they induce more stretching of the suspended cell membrane, thus deteriorating cell functions and viability. Due to this fact, even if the material surface does not show high roughness, the response of cell adhesion and morphology to nanotopography could be essential and connected with cell types.

Microscopic observations of the samples (***Figure 4***) revealed their surface to be smooth. However, at a smaller magnification (data not shown) distinct marks were seen – the imprint of the moulding form. Profilometric observations confirmed the microstructural examinations. Bigger numbers of silver nanoparticles were visible on the surface of the incubated samples. Such a phenomenon was consistent with the roughness parameters obtained. It was also observed that marks imprinted by the moulding form were more noticeable. Silver agglomerates were visible on both the surface and cross-sections of the samples– a direct reason for the roughness increase.

SEM images of osteoblast cells (***Figure 5***) revealed the characteristic proper morphology and homogeneous distribution on the polymer and composite material surfaces, with no tendency to form clusters. Silver nanoparticles did not seem to influence cell adhesion to the polymer surfaces. The results obtained proved similar cell proliferation levels for all materials tested. It has also been reported that the nanoscale roughness and stiffness of biomaterials are two major independent physical factors that can dictate the long-term function of osteoblasts [32]. On the one hand the nanoscale island pattern provides greater osteoblast adhesion than those obtained with the nanoscale pit pattern and microscale island [33]. But on the other hand, increased nanoscale roughness may hinder cell proliferation because of the too strong adhesion of osteoblasts [34]. A change in nanometer roughness is also related to changes in wettability [35]. Webster et al. speculated that hydrophilicity is enhanced on Ti with nanometer surface features due to the increased presence of surface defects as compared to conventional Ti [36]. Therefore we concluded that osteoblast differentiation and proliferation may be controlled by surface parameters such as roughness and wettability. Keeping a balance between those two parameters does not influence the adhesion of osteoblastic cells.

Conclusions

In this work, ABS (poly)acrylonitrile butadiene styrene copolymer was examined to assess the impact of the surface characteristics on the biomaterial's interaction with the surrounding environment. The study's findings revealed hydrophilic characteristics of the samples investigated. Profilometry examinations disclosed an overall low roughness, with spots of higher roughness. After 6-month incubation in deionised water, the samples showed slightly higher levels of roughness. SEM examinations confirmed

a homogeneous distribution of nanosilver in the polymer matrix both on the surface and inside the samples, as well as the presence of pores and spherulites. After incubation, the spots with nanosilver became more visible on the sample surface, proving an increase in the surface roughness. On the basis of the results obtained, it may be concluded that the parameters of the biomaterial surface play a crucial role in its interaction with the biological environment. Selecting proper characteristics ensures implant biostability, thus preventing its rejection. Microscopic assessment of the cells in direct contact with the samples proved the cytocompatibility of the biomaterials investigated.

Acknowledgements

This work was financially supported by the National Centre for Research and Development grant no: LIDER/ 154/L-6/14/ NCBR/2015.

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Received 27.04.2018 Reviewed 11.09.2018