SURFACE MODIFICATIONS OF BIOMATERIAL WITH DIFFERENT COLD PLASMA REACTORS TO IMPROVE CELL ADHESION

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Abstract

There is a growing trend in the engineering of biomaterials, focusing on surface modifications of biomaterials to improve their mechanical strength, corrosion resistance, and biological properties. Cold plasma treatment may improve biological properties of biomaterials for biomedical applications by enhancing their integration with host tissue. This study investigated the influence of different cold plasma treatments on the surface properties of a polysaccharides-based biomaterial to improve cell adhesion to its surface. The samples were subjected to plasma treatment using three different reactors operating at atmospheric pressure: gliding arc discharge (GAD) reactor, dielectric barrier discharge (DBD) plasma jet, and DBD surface reactor. Next, surface chemistry of the biomaterial after plasma treatment was determined by ATR-FTIR analysis. Furthermore, a cell adhesion assay on the samples was carried out using normal human skin fibroblasts (BJ cell line). The attenuated total reflection Fourier transform infrared analysis (ATR-FTIR) showed that new potential functional groups could be formed on the material surface after plasma treatment. However, plasma treatment of the samples did not enhance cell adhesion to the surface of the polysaccharides-based biomaterial. Thus, the obtained results indicate that plasma treatment using GAD reactor, DBD plasma jet, and DBD surface reactor was not effective for surface modification and cell responses.

Keywords: *curdlan, agarose, gliding arc discharge reactor, dielectric barrier discharge plasma jet, dielectric barrier discharge surface reactor, surface modification*

Introduction

Nowadays, there is a growing trend in the engineering of biomaterials that focuses on surface modifications to improve mechanical strength, corrosion resistance, and biological properties of the implants, e.g. to increase cell adhesion and proliferation and reduce the risk of infection after implantation [1,2]. Surface roughness, wettability, surface chemistry, and charge are among the factors that influence cell adhesion [2,3]. Moreover, strong adhesion is crucial for rapid cell proliferation and migration on the surface of biomaterials used as potential implants [3]. It was proven that polar and positively charged surfaces provide the most effective adsorption of protein to the surface of biomaterials, allowing good cell adhesion. For instance, Keselowsky et al. reported that the adhesion of cells to surfaces with differently chargeable functional groups followed the trend: OH > COOH = NH_2 > CH_3, by modulating fibronectin adsorption and direct integrin binding of cells to the fibronectin [3,4]. Plasma techniques are well-established technologies commonly used to modify the chemistry and topography of the biomaterials for different applications to improve their biocompatibility and interactions with tissues. Plasma techniques may be classified into two main classes: thermal plasma that is used mainly as a surface coating technology and low-temperature plasma that is used to directly treat living tissues [1,2]. Electric plasmas depending on the used substrate gas, allow formation of highly reactive species, such as ions, electrons, photons, free radicals, etc., which may further react with the treated material [5,6].

The aim of this work was to evaluate the impact of different cold plasma treatments, using 3 reactors operating at atmospheric pressure: gliding arc discharge (GAD) reactor, dielectric barrier discharge (DBD) plasma jet, and DBD surface reactor, on the improvement of the surface of biomaterials to increase cell adhesion. In the study, a polysaccharides-based biomaterial containing curdlan and agarose was used, whose surface has previously been proven to be unsupportive to cell adhesion [7]. After the plasma treatment, the surface chemistry of the biomaterial was assessed by ATR-FTIR. Moreover, the evaluation of cell viability and adhesion to the surface of plasma-treated biomaterial was performed.

Materials and Methods

Preparation of biomaterial

The biomaterial composition was previously optimized to achieve the most desired microstructural and physicochemical properties. The resultant curdlan/agarose biomaterial and method for its production were claimed in the Polish Patent no. 236367 (2021). Briefly, the biomaterial was prepared by suspending 2% (*w/v*) curdlan (Wako Pure Chemicals Industries, Japan) and 2% (*w/v*) agarose (Sigma-Aldrich Chemicals, Poland) in deionized water. The suspension was then transferred to round-shaped flat molds, which were placed in a water bath at a temperature of 95°C for 20 min, and then the resultant biomaterials were cooled and frozen at -80°C for 24 hours. The frozen samples were lyophilized for 18 h under medium vacuum of 6 x 10^{-2} mbar (LYO GT2-Basic, SRK Systemtechnik GmbH, Riedstadt, Germany).

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Plasma generator and plasma treatment of the surface of biomaterials

The plasma treatment was performed using three reactors operating at atmospheric pressure: gliding arc discharge (GAD) reactor, dielectric barrier discharge (DBD) plasma jet, and DBD surface reactor. Their operating parameters are summarized in TABLE 1, and the discharge geometry is presented in FIG. 1. For each reactor, 3 series of repetitions were performed for the treatment times of 30, 120 and 300 s. The treatment time and distance were selected as the most promising on the basis of the former research work using DBD and GAD reactors [5,8].

Surface chemistry analysis of biomaterial after plasma treatment

In the ATR-FTIR study on the identification of bonds after plasma treatment, a FT-IR-4200 type A (Jasco, Tokyo, Japan) spectrometer with ATR PRO ONE (Jasco, Tokyo, Japan) single reflection attachment with ZnSe crystal was used. The measurements were taken immediately after the treatment, using a set screw with a torque limiter.

Evaluation of cell viability and adhesion to the surface of biomaterials after plasma treatment

Cell culture experiments were performed using normal human skin fibroblasts (BJ cell line) obtained from the American Type Culture Collection (ATCC-LGC Standards). The BJ cells were incubated at 37°C in a humidified atmosphere (95%) with 5% carbon dioxide content and maintained in Eagle's Minimum Essential Medium (EMEM, ATCC-LGC Standards, Teddington, UK) with 10% fetal bovine serum (Pan-Biotech GmbH, Aidenbach, Bavaria, Germany), and 1% penicillin-streptomycin solution (Sigma-Aldrich Chemicals, Poland).

Before the experiment, the cube-shaped biomaterials (3 mm x 3 mm x 3 mm) were placed in 48-well plates and preincubated overnight in 300 µl of the EMEM complete culture medium. Then, 1 x 10^5 BJ cells (at passage 6) were seeded on the biomaterials in the 500 µl culture medium and cultured at 37°C for 48 hours. To evaluate cell viability and adhesion to the surface of biomaterials, the BJ cells were stained using Live/Dead Double Staining Kit (Sigma-Aldrich Chemicals, Poland) and visualized using a confocal laser scanning microscope (CLSM, Olympus Fluoview equipped with FV1000, Olympus, Japan).

TABLE 1. Characteristics of plasma reactors.

Type	Max. voltage	Mean power	Frequency	Working gas	Geometry
GAD	3.7 kV	40 W	50 Hz	Forced nitrogen flow (440 lph)	Sample placed perpendicular to the gas stream, 2 cm from the electrodes.
Surface DBD	3.7 kV	10 W	17 kHz	Air	Sample placed at a distance of 2 cm from the electrode, in a 37 dm^3 glass cubic container.
DBD plasma jet	3.7 kV	6 W	17 kHz	Forced flow of a mixture containing helium (100 lph) and nitrogen (1 lph)	Sample placed perpendicular to the gas stream, 3 cm from the end of ceramic tube

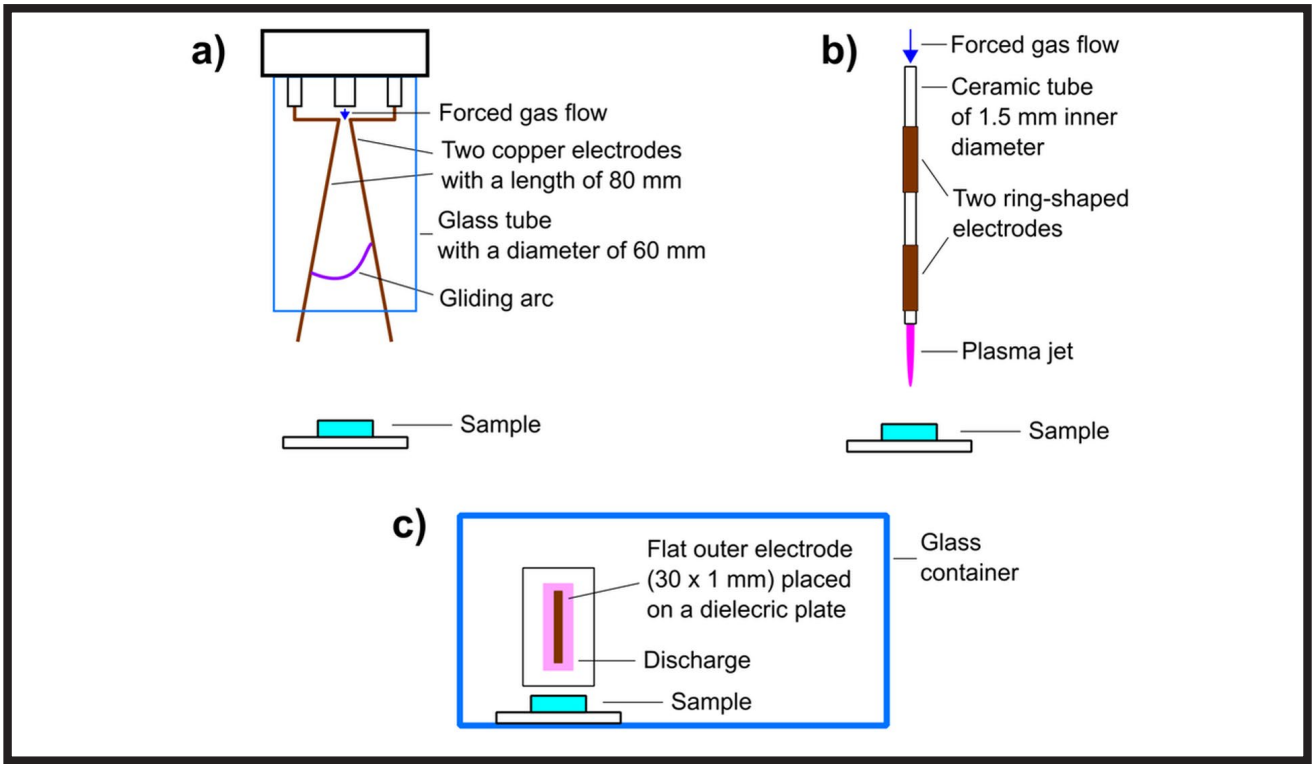

FIG. 1. Schematic diagrams of the atmospheric plasma treatment systems: (a) GAD reactor; (b) DBD plasma jet; (c) surface DBD.

Results and Discussions

Physicochemical analysis of the surface of biomaterials after plasma treatment

The ATR-FTIR analysis was carried out for carbon and nitrogen bond matching. The presented results were characterized by a low value of the signal to noise ratio, which, combined with the presence of many other peaks in the studied areas, did not allow to clearly indicate the exact formation of permanent bonds. The noticeable changes caused by plasma treatment were best seen for the band in the range from 1300 to 1900 cm^{-1}, where peaks characteristic for ketones (bands 1710-1720, 1680-1700, 1715-1810 cm^{-1}) and nitro compounds (bands 1500-1600, 1300-1390 cm^{-1}) were observed (FIG. 2). Peaks associated to C=O stretching (potentially of ketones or amides) around 1640 and 1680 cm^{-1}; N-H bending of amines around 1650 and 1340 cm^{-1} and some peaks associated with both symmetrical and asymmetrical N-O stretching around 1583 and 1350 cm^{-1} could be noticed. The measurements were carried out just after the plasma treatment and changes in spectra appeared for all the tested cases, including reactors and treatment times (FIG. 2), in comparison to the untreated material. As expected, the absorbance value increased with the treatment time. The highest absorbance values indicating potential formation of new functional groups, which might improve biocompatibility of plasma treated material were obtained for the materials treated with the surface DBD reactor. The absorbance value for the mentioned peaks was on average 58% higher than the GAD reactor and 157% higher than the DBD plasma jet. Unfortunately, such high absorbance did not find reflection during the evaluation of cell viability and adhesion to the surface, which may suggest that the 300 s treatment time was too short to allow the formation of permanent bonds. Moreover, some secondary reactions could take place during the transportation and preservation of samples, which effected in further regrouping, leading to the loss of surface functionalization. Thus, further investigations employing changing of plasma treatment parameters are planned.

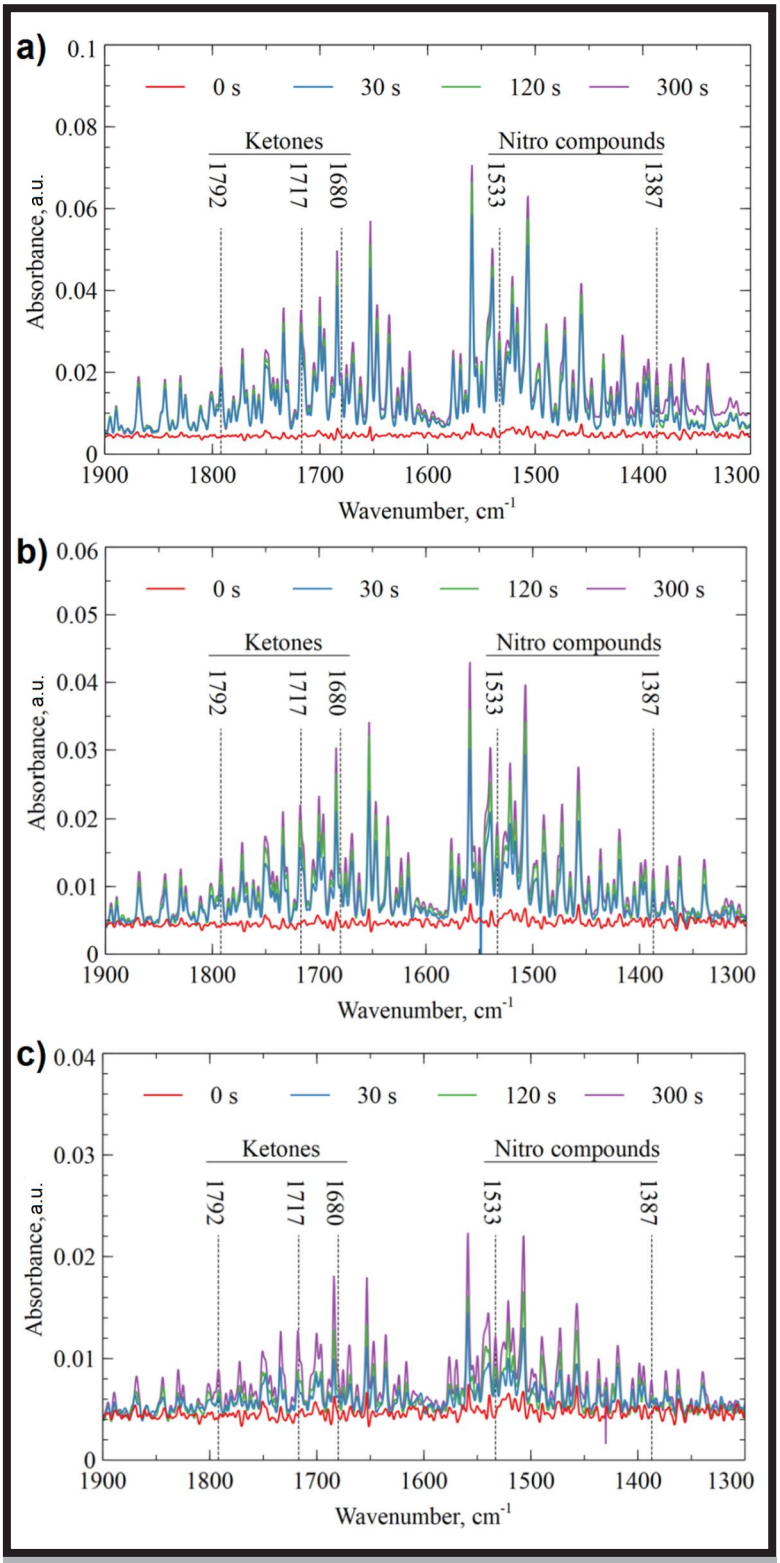

FIG. 2. ATR-FTIR spectra of the curdlan/agarose biomaterials after different plasma treatment times with (a) the DBD surface reactor, (b) the GAD reactor, and (c) the DBD plasma jet reactor.

Evaluation of cell viability and adhesion to the surface of biomaterials after plasma treatment

Polysaccharides are often used in various tissue engineering applications for the production of implants or artificial organs. Natural polymers, such as curdlan (linear bacterial β-1,3-glucan) and agarose, are characterized by high biocompatibility, biodegradability, and wide availability making them widely used in biomaterials engineering [9]. In our previous research [7], a curdlan/agarose biomaterial was developed which was characterized by non-cytotoxicity and a foam-like structure with superabsorbent ability. Moreover, the surface of the fabricated biomaterial was unfavourable for cell adhesion. In this study, the cold plasma treatment of the developed biomaterial using different reactors was performed to improve cell adhesion to its surface. As shown in FIG. 3, the plasma treatments of the surface of polysaccharides-based biomaterial did not improve cell adhesion.

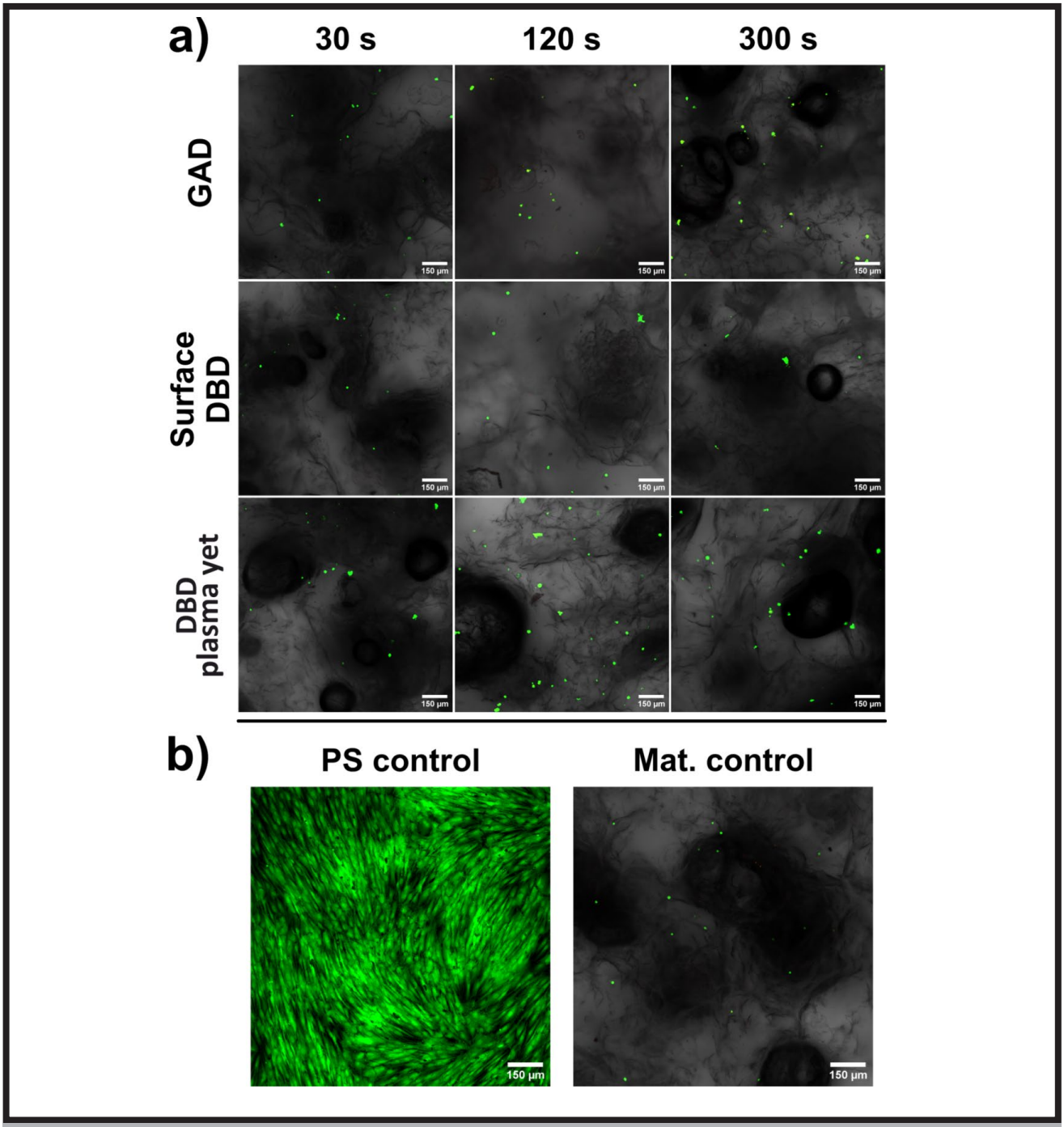

FIG. 3. Evaluation of cell viability and adhesion to the surface of (a) plasma-treated biomaterials and (b) non-treated biomaterial (Mat. Control) by Live/Dead double fluorescent staining and confocal laser scanning microscope visualization (PS control – control cells cultured on the surface of polystyrene well of multiwell plate; viable cells – green fluorescence; dead cells – red fluorescence; magnification 100x, scale bar = 150 µm).

The results obtained with Live/Dead staining of BJ cells cultured on the surface of biomaterials after the plasma treatment showed that the cells were viable (only green fluorescence was detected), but had a round shape, meaning that surfaces of biomaterials did not support cell attachment and adhesion. The extended plasma treatment times (from 30 to 300 s) were not effective for improvement of cell responses either. Moreover, there were no differences between the morphology of the cells cultured on the surface of the plasma-treated biomaterials and the non-treated control biomaterial (Mat. control).

Conclusions

Plasma treatment is widely used to improve cell interactions with polymer surfaces used in biomedical applications [10-12]. In this study, it was proven that the plasma treatment of the curdlan/agarose biomaterial using GAD reactor, DBD plasma jet, and DBD surface reactor was not effective for the surface modification and cell responses. Human fibroblasts cultured on the surface of biomaterials after plasma treatment had a round shape, indicating that the biomaterial surface did not support cell adhesion. Thus, further studies are needed to find an efficient plasma method that will improve cell adhesion to the curdlan/agarose biomaterial.

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References

[1] Kazimierczak P., Przekora A.: Osteoconductive and osteoinductive surface modifications of biomaterials for bone regeneration: A concise review. Coatings 10(10) (2020) 971. doi:10.3390/coatings10100971

[2] Yan M., Hartjen P., Gosau M., et al.: Effects of a novel cold atmospheric plasma treatment of titanium on the proliferation and adhesion behavior of fibroblasts. Int J Mol Sci. 23(1) (2022) doi:10.3390/ijms23010420

[3] Przekora A.: The summary of the most important cell-biomaterial interactions that need to be considered during in vitro biocompatibility testing of bone scaffolds for tissue engineering applications. Mater Sci Eng C. 97 (2019) 1036-1051. doi:10.1016/j.msec.2019.01.061

[4] Keselowsky B.G., Collard D.M., García A.J.: Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion. J Biomed Mater Res Part A. 66(2) (2003) 247-259. doi:10.1002/jbm.a.10537

[5] Pawłat J., Terebun P., Kwiatkowski M., et al.: Evaluation of oxidative species in gaseous and liquid phase generated by mini - gliding arc discharge. Plasma Chem Plasma Process. 39(3) (2019) 627-642. doi:10.1007/s11090-019-09974-9

[6] Przekora A., Audemar M., Pawlat J., et al.: Positive effect of cold atmospheric nitrogen plasma on the behavior of mesenchymal stem cells cultured on a bone scaffold containing iron oxide-loaded silica nanoparticles catalyst. Int J Mol Sci. 21 (2020) 4738. doi:10.3390/ijms21134738

[7] Wojcik M., Kazimierczak P., Benko A., Palka K., Vivcharenko V., Przekora A.: Superabsorbent curdlan-based foam dressings with typical hydrocolloids properties for highly exuding wound management. Mater Sci Eng C 124 (2021) 112068. doi:10.1016/j.msec.2021.112068

[8] Kwiatkowski M., Terebun P., Mazurek P., Pawlat J.: Wettability of polymeric materials after dielectric barrier discharge atmospheric--pressure plasma jet treatment. Sensors Mater. 30(5) (2018) 1207-1212. doi:10.18494/SAM.2018.1890

[9] Zarrintaj P., Manouchehri S., Ahmadi Z., et al.: Agarose-based biomaterials for tissue engineering. Carbohydr Polym. 187 (2018) 66-84. doi:10.1016/j.carbpol.2018.01.060

[10] Liu C., Bai J., Wang Y., et al.: The effects of three cold plasma treatments on the osteogenic activity and antibacterial property of PEEK. Dent Mater. 37(1) (2021) 81-93. doi:10.1016/j.dental.2020.10.007

[11] Teraoka F., Nakagawa M., Hara M.: Surface modification of poly(L-lactide) by atmospheric pressure plasma treatment and cell response. Dent Mater J. 25(3) (2006) 560-565. doi:10.4012/dmj.25.560

[12] Hage M., Khelissa S., Akoum H., Chihib N.E., Jama C.: Cold plasma surface treatments to prevent biofilm formation in food industries and medical sectors. Appl Microbiol Biotechnol. 106(1) (2022) 81-100. doi:10.1007/s00253-021-11715-y