GROWTH AND OSTEOGENIC DIFFERENTIATION OF HUMAN OSTEOBLAST-LIKE CELLS ON NANOFIBROUS SCAFFOLDS LOADED WITH DIAMOND NANOPARTICLES: IMPROVEMENT OR IMPAIRMENT?

JANA MUSILKOVA[1], LUBICA STANKOVA[1], STEPAN POTOCKY[2], ALEXANDER KROMKA[2], DENISA STRANSKA[3], LUCIE BACAKOVA[1]

[1] INSTITUTE OF PHYSIOLOGY, ACADEMY OF SCIENCE OF THE CZECH REPUBLIC, CZECH REPUBLIC
[2] INSTITUTE OF PHYSICS, ACADEMY OF SCIENCE OF THE CZECH REPUBLIC, CZECH REPUBLIC
[3] INSTAR TECHNOLOGIES, JOINT-STOCK CO, CZECH REPUBLIC
*E-MAIL: JANA.MUSILKOVA @FGU.CAS.CZ

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Introduction

Nanofibrous scaffolds loaded with diamond nanoparticles (DNPs) are considered as a promising materials for engineering of various types of tissues, including bone tissue. In our earlier studies, nanocrystalline diamond films proved as excellent substrates for the adhesion, growth and osteogenic differentiation of human-bone derived cells, particularly after their doping with boron [1] or termination with oxygen [2]. In the present study, we focused on the cell behaviour of human osteoblast-like Saos-2 and MG 63 cells on PLLA membranes loaded with DNPs prepared by detonation. The obtained results were compared with those in our previous studies, i.e., with the cell behavior on PLGA membranes with DNPs prepared by RF-PACVD method [3,4].

Materials and Methods

Detonation diamonds (NanoAmando, Nanocarbon Research Institute Co., Ltd. Japan) were added on nanofibrous membranes prepared by needle-less electrospinning technique in 6 concentrations ranging from 0.02 to 0.7 g per 100 ml of the polymer solution. After evaporation of the solvent, the concentration of DNPs ranged from 0.44 to 12.28 wt. %. Scaffolds were well characterized by Scanning electron microscopy, IR spectroscopy, Raman spectroscopy, XPS analysis and water drop contact angle.

The scaffolds were seeded by human Saos-2 and MG 63 cells (11 000 cells/cm²) and cultivated at 37°C in a humidified air atmosphere containing 5% of CO_2. We performed the LIVE/DEAD test, ELISA, MTT metabolic test and Real Time PCR to estimate the cell adhesion, viability, growth and metabolic activity of cells on the scaffolds, concentration of specific markers of cell adhesion, osteogenic cell differentiation, cell cycle regulation and apoptosis at the protein as well as at mRNA level. Data were analyzed using ANOVA, Student-Newman-Keuls Method. Statistical significance $p \leq 0.05$.

Results and Discussion

We found that the increasing concentration of DNPs in PLLA nanofibrous scaffolds has rather negative effects on the cell adhesion, viability, metabolic activity (FIG. 1), growth and osteogenic differentiation of MG 63 and Saos-2 cells. In some cases, we observed a slight improvement of the cell behavior on the scaffolds with medium DNP concentrations (1.72 to 3.38 wt. %). These results differ from the results obtained in our previous studies, employing DNPs prepared by radiofrequency PACVD method, In these studies, the cell adhesion and growth on DNP-loaded scaffolds were either unchanged (in MG 63 cells) or even improved (in human bone marrow mesenchymal stem cells) in comparison with the pure polymeric scaffolds [3,4].

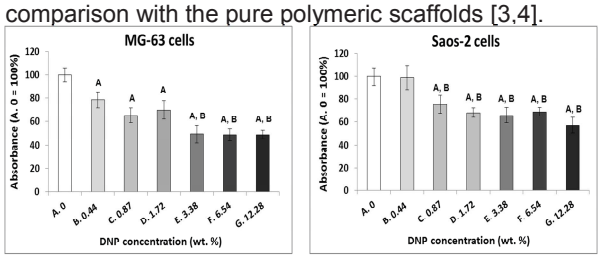

FIG. 1. The mitochondrial activity of human osteoblast-like MG-63 and Saos-2 cells, measured by XTT test on day 3 after seeding on PLLA nanofibrous membranes loaded with 0 to 12.28 wt% of DNPs. Absorbances are given in % of values obtained from pure PLLA membranes (sample A.0).

Consecutively we focused our interest on gene expression of the following markers associated with the regulation of cell cycle and apoptosis: cyclin D, a member of the cyclin protein family that is involved in regulating cell cycle progression; survivin, an inhibitor of caspase activation; Bcl-2 (B-cell lymphoma 2), an important anti-apoptotic protein and oncogene; and KLF6 (Krueppel-like factor 6), a transcription factor involved in growth-related signal transduction, cell proliferation and differentiation, development, apoptosis and angiogenesis, postulated as a tumor suppressor. The expression of cyclin D (FIG. 2), and survivin in Saos-2 cells fell down remarkably with increasing DNP concentration, while the expression of the anti-apoptotic protein Bcl-2 and (KLF6) rose significantly in cells on the scaffolds with lower DNP concentrations (Bcl-2: up to 0.87 wt.%, KLF6: up to 0.44 wt.%), and then decreased. The response obtained in MG 63 cells was weaker.

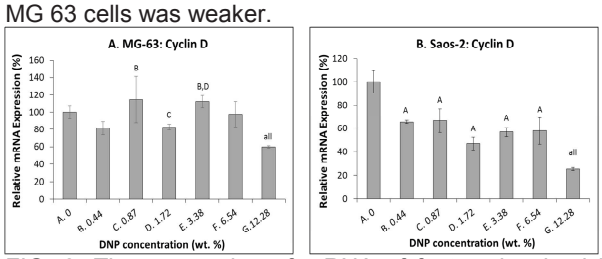

FIG. 2. The expression of mRNA of factors involved in cell cycle progression (Cyclin D) test on day 14 after seeding.

Conclusions

The detonation DNPs might have a direct toxic influence on cells. Thus, the mode of preparation and properties of diamond nanoparticles are important for their biocompatibility and for their applicability in bone tissue engineering.

Acknowledgments

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