The influence of semiconductor nanoparticles upon the activity of mesenchymal stem cells

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Abstract— **In this paper, we report on the viability and proliferation of mesenchymal stem cells after exposure to different types of semiconductor nanoparticles. The nanoparticles used for the tests are based on GaN thin layers grown on commercial ZnO and $ZnFe_2O_4$ nanoparticles. Different quantities of nanoparticles incubated with mesenchymal stem cells influence the metabolic activity of cells, which was assessed by the MTT assay. The cytotoxic effect of ZnO nanoparticles on MSC was demonstrated and no harmful effect of the other materials.**

Keywords— **nanoparticles, gallium nitride, mesenchymal stem cells, MTT.**

I. Introduction

Stem cells represent unspecialized cell type that can differentiate into various specialized cell types under certain physiological or experimental conditions. The mesenchymal stem cells are widely used in cell therapy with a different success-rate [1]. In order to increase the impact, interdisciplinary research that implies the use of nanoparticles in biomedical applications, like bio-sensing, imaging, or therapy is ongoing [2-7]. The use of nanoparticles in medical applications is widely investigated through different approaches. Nanomedicine is also oriented to cancer treatment with stem cells utilization as drug carriers [8]. One of the actual problems in nanomedicine is the evaluation of nanoparticles cytotoxicity and their potential impact at the cellular level. Our previous investigations have shown that uncoated gallium nitride nanoparticles (GaN) do not affect the viability and proliferation of endothelial cells [9] and can be used for multifunctional therapeutic purposes which include cells spatial redistribution [10].

In this work, we report on the interaction of rat MSC with different types of nanoparticles. We used both commercially available zinc oxide (ZnO) and zinc ferrite ($ZnFe_2O_4$) as well as synthesized GaN-based nanoparticles. The metabolic activity of cells after exposure to nanoparticles was assessed using the MTT assay, which shows a highly toxic effect of the ZnO nanoparticles and no harmful effect of the other materials.

II. Experimental

Nanoparticles synthesis and characterization

Nanometre-scale thin layers of GaN have been grown on sacrificial zinc ferrite ($ZnFe_2O_4$) based nanoparticles acquired from Sigma-Aldrich (CAS#12063-19-3). The growth took place in a horizontal hydride vapor phase epitaxy (HVPE) reactor with four temperature zones. In the source zone, at T=850°C, the GaCl is formed after the interaction of HCl with metallic Ga. Then, in the reaction zone at 600°C, the formed GaCl interacts with NH_3 for 10 min in

order to initiate the GaN growth on the $ZnFe_2O_4$ nanoparticles. The GaN layer growth accompanied by simultaneous reduction of ZnO in the sacrificial substrate nanoparticles occurs at 800°C for 10 min in the H_2 flow rate of 3.6 l/min. During the growth process, the ammonia and hydrogen chloride flow was kept constant at 500 ml/min and 15 ml/min, respectively. The initial nanoparticles, as well as the resulted material after the GaN growth, have been characterized using the electron microscopy tools, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Mesenchymal Stem Cells isolation and culture

The isolation, culture, and use of rat MSC in the research activities were approved by the Ethics Committee of the Moldovan State University of Medicine and Pharmacy "Nicolae Testemitanu" on 18.06.2015.

The MSC were isolated from the bone marrow of 5 months old Wistar male rat. After rat euthanasia, the bone marrow from long tubular bones has been flushed with warm PBS (HiMedia, India). The suspension was centrifuged for 10 min at 170 *g*, followed by MSC isolation in mesenchymal stem cells expansion medium HiMesoXL (HiMedia, India) supplemented with antibiotics and antimycotics. The incubation was performed in 25 cm^2 cell culture flasks (Nunc, Denmark) at 37°C with 5% CO_2. The cells were cultured in 2 passages followed by cryopreservation by $5x10^5$ cells/ml in FBS (Lonza, Belgium) with 10% DMSO (OriGen Biomedical, Germany). The MSC isolation and identification was done following the chondrocytes line differentiation protocol [9].

To perform the experiment, $5x10^5$ MSC were cultured in 75 cm^2 culture flasks (Nunc, Denmark) with 15 ml DMEM/Ham's F-12 medium (Sigma, UK) supplemented with 10 % FBS (Lonza, Belgium) and antibiotic-antimycotic solution. The medium was completely changed every 2 days until the culture gained 80-90% of cells confluence. After trypsinization, the cells were counted in hemocytometer with Trypan blue exclusion, followed by cells seeding at a density of $1x10^4$ cells/ml in 24 well tissue culture test plates (TPP, Switzerland) for MTT cell viability assay.

The suspension of MSC was prepared using nanoparticles at a concentration of $1x10^4$ cells/ml with each type of nanoparticles at a concentration of 50 ng/ml, 25 ng/ml and 10 ng/ml. One milliliter of each nanoparticle-MSC suspension type was moved in wells with glass coverslips (n=3), and incubated at 37°C with 5% CO_2, for 2 days under magnetic field influence.

MTT assay

The MTT assay started 24 h after the medium supplemented with nanoparticles was added to 24 well tissue culture test plates seeded with MSC and has been performed every day during the incubation period (n=3). The culture medium was replaced by 1ml of 2.5 mg/ml MTT (Sigma, UK) solution prepared in DMEM/Ham`s F-12 medium (Sigma, UK), followed by two hours incubation at 37°C with 5% CO_2. After incubation, the MTT solution was replaced by 1 ml of 99,8% isopropanol (STANCHEM, Poland). The plates covered with tinfoil have been shaken for 15 min at 100 rpm (ES-20, Biosan), followed by the color change quantification using the plate reader (Synergy H1, BioTek) at 570 nm.

The cell viability was assessed by the formula:

$$Cell\ viability\ (\%) = \frac{(\mathrm{OD}_{570}\ \text{of test nanoparticles}) - (\mathrm{OD}_{570}\ \text{of blank})}{(\mathrm{OD}_{570}\ \text{of control}) - (\mathrm{OD}_{570}\ \text{of blank})} x\ 100\% \quad (1)$$

Cells preparation for scanning electron microscopy

The morphology of rat MSC cultivated in the presence of nanoparticles exposed to the magnetic field was studied using a Vega Tescan SEM. Before imaging, the cells were fixed in glutaraldehyde, dehydrated with ethanol, dried, and covered with a thin gold (Au) layer in order to avoid charging effects during electron microscopy scanning. The fixation process was done at 4°C in 2.5 % glutaraldehyde for 12 h, followed by other 24 h in a saline buffered solution (NaCl 0.9 %). The dehydration process involved incubation in gradually increasing ethanol concentrations from 30% to 97% at room temperature. Since we were more interested in seeing the cells redistribution under magnetic field influence rather than the cell membrane integrity, the drying process has been performed in a normal atmosphere at room temperature. Before imaging, the samples were coated with an ultrathin layer of Au by using a Cressington 108 auto sputter coating machine.

III. RESULTS AND DISCUSSION

Figure 1 shows the SEM images of the initial commercial nanoparticles and their morphology after GaN growth. During the epitaxial growth process, the sacrificial layers of ZnO and $ZnFe_2O_4$ are decomposed due to high temperature and the harsh environment in which GaN is grown. The chemical composition and crystalline structure have been analyzed in our previous works [10, 11].

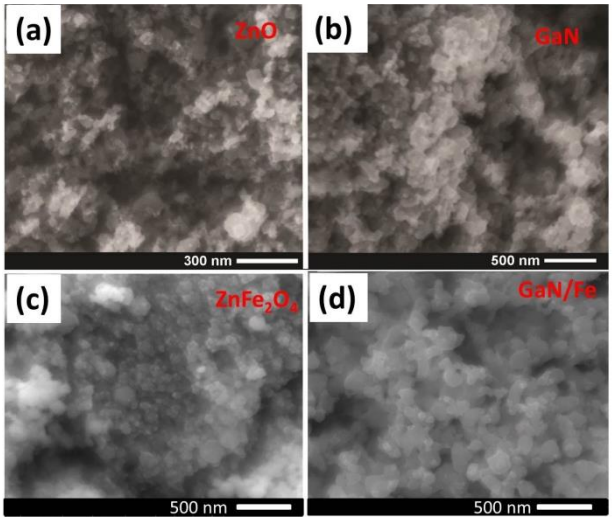

Fig.1. SEM images of nanoparticles. (a) ZnO, (b) GaN/ZnO, (c) $ZnFe_2O_4$ and (d) GaN/Fe.

The influence of different types of nanoparticles has been assessed by incubating them in different quantities with living cells. Figure 2 depicts the scanning electron micrographs (SEM) of MSC after three days of incubation with 50µg/ml of different types of nanoparticles. According to the pictures, one can observe the tendency of cells to collect the nanoparticles and the clusters of nanoparticles from the media and deposit in vesicles. The morphology of cells seems not to be affected by GaN-based nanoparticles, while there are no attached cells in the samples incubated with zinc oxide nanoparticles even at concentrations as low as 25 µg/ml

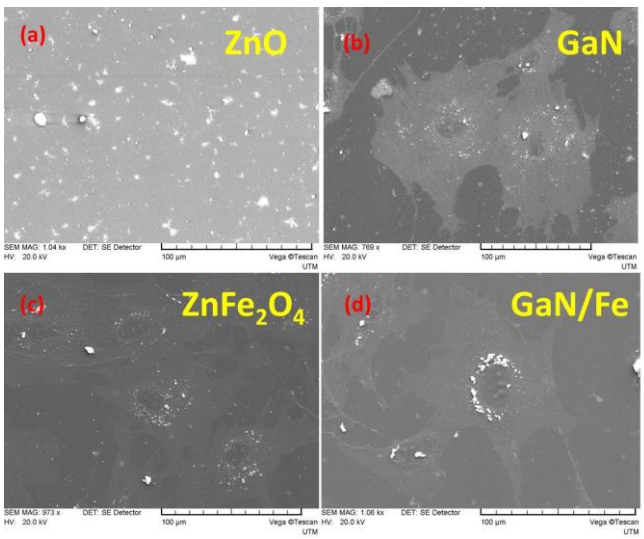

Fig. 2. SEM images of MSC after three days of incubation with 50µg/ml of (a) – ZnO, (b) – GaN, (c) – $ZnFe_2O_4$ and (d) – GaN/Fe nanoparticles

The MTT assay confirms that ZnO becomes highly toxic at higher concentrations, while the other types of nanoparticles are being accepted and uptaken by MSC. Figure 3 presents the dynamic evolution of cells activity during the incubation process with different concentrations of nanoparticles based on different semiconductor materials. One can notice a time-dependent decreasing tendency in cells metabolic activity for all the nanoparticles used. The concentration-related tendency is also noticeable, and the concentration of 50 µg/ml of nanoparticles incubated for three days leads to a decrease in cells metabolic activity with about 50 % comparing to the control group.

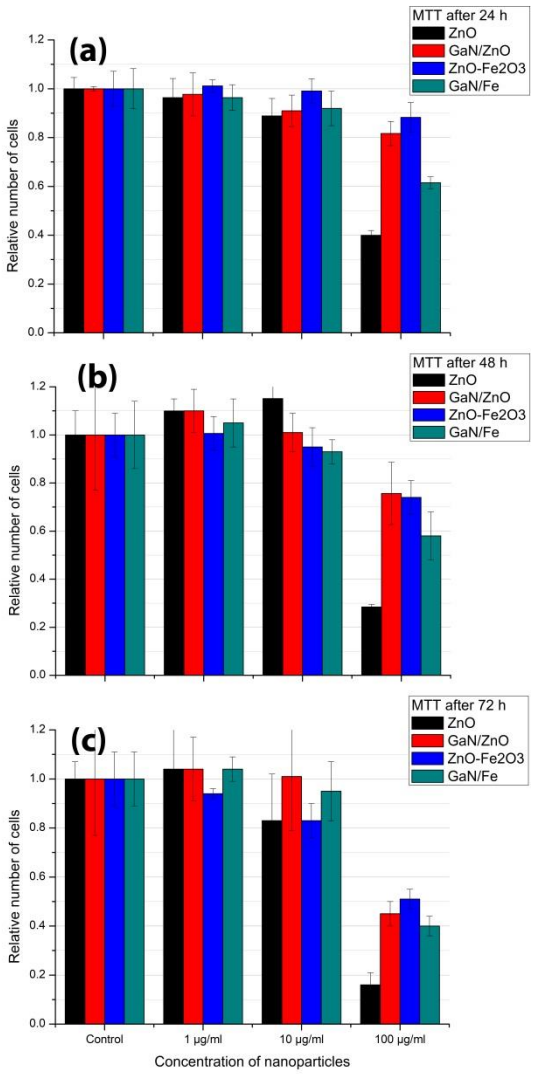

Fig. 3. MTT product after (a) 24 h, (b) 48 h and (c) 72 h of incubation of cells with 10, 25 and 50 µg/ml of nanoparticles.

The high toxicity of ZnO nanoparticles is attributed to poor chemical stability of the material: the Zn^{2+} ions concentration increases in the growth medium, which lead to cells apoptosis. The GaN nanoparticles grown on ZnO and ZnFe2O4 nanoparticles stabilize the remaining oxide layer and increase the material chemical stability and thus are less toxic for living cells.

CONCLUSIONS

The activity of mesenchymal stem cells is higly affected by ZnO nanoparticles with dimensions less than 50 nm at a concentration higher than 25 μg/ml. The GaN based nanoparticles does not affect the activity of MSC significantly at low concentrations, while at higher concentrations (> 50μg/ml) the metabolic activity of MSC is inhibited by the presence of nanoparticles.

ACKNOWLEDGMENT

The authors acknowledge the support from the Ministry of Education, Culture and Research of the Republic of Moldova under the Grants #18TC, #15.817.02.29A and #15.817.04.04F. This work has received partial funding from the European Union's Horizon 2020 research and innovation programme under grant #810652 (NanoMedTwin project).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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