Nanoparticles for Cells Proliferation Enhancement

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***Abstract* — The potential of semiconductor nanoparticles as stimulator for avian mesenchyme stem cells proliferation enhancement is demonstrated. The effect is related to nanoparticles polarization due to external ultrasound field resulting in local electrical stimulation.**

Our preliminary results demonstrates that the number of cells have been increased by 23 % (± 2%) in cell cultures under the action of external ultrasound stimulation. Morphological analysis and viability shows no differences between the control group and the group studied. These results suggest the possibility for tissue regeneration enhancement by remote stimulation of implanted semiconductor nanoparticles

I. INTRODUCTION

The modern theory of tissue healing is based on growth factors interactions and biochemistry. The actions of cytokines and growth factors are said to initiate and mediate the various stages of inflammation and repair that normally follow tissue damage. Yet evidence which has accumulated over many decades suggests that a full description of the physiology of healing must also include the role of bioelectricity, that are an accumulation and flow of charge that are generated endogenously, within the body. The importance of bioelectricity in functions such as nervous system signalling and muscle contraction has been long appreciated, but it is also involved in many other physiological processes. These include the development, adaptation, repair and regeneration of tissues throughout the body [1, 2, 3, 4].

Materials for bioaplications must posses a series of properties like biocompatibility, minimum toxicity and others. GaN semiconductor is known as a chemically stable material and already demonstrated its applicability for different biosensors [5, 6, 7].

Another important merit of GaN is the pronounced piezoelectric property that already found implementation in MEMS&NEMS [8, 9].

For the healing the most important are the mesenchymale stem cells, which assure the tissue regeneration, and the recovery of the organ integrity, this way, we attended to demonstrate that the generation of an low bioelectrical field based on the piezoelectric effect of GaN nanoparticles can induce a higher proliferation rate in mesenchymale stem cell cultures. The proposed technique for improving cells proliferation improvement involves both, biocompatibility and piezoelectric properties of GaN for remote electrical stimulation using nanoparticles. When GaN nanoparticles are placed in ultrasound field, the piezoelectric effect leads to nanostructures polarization which results in local cell electrical stimulation (fig.1). The effect of electrical stimulation is a well known method for tissue regeneration enhancement [10] being related to cells ions currents redistribution thus leading to proliferation improvement.

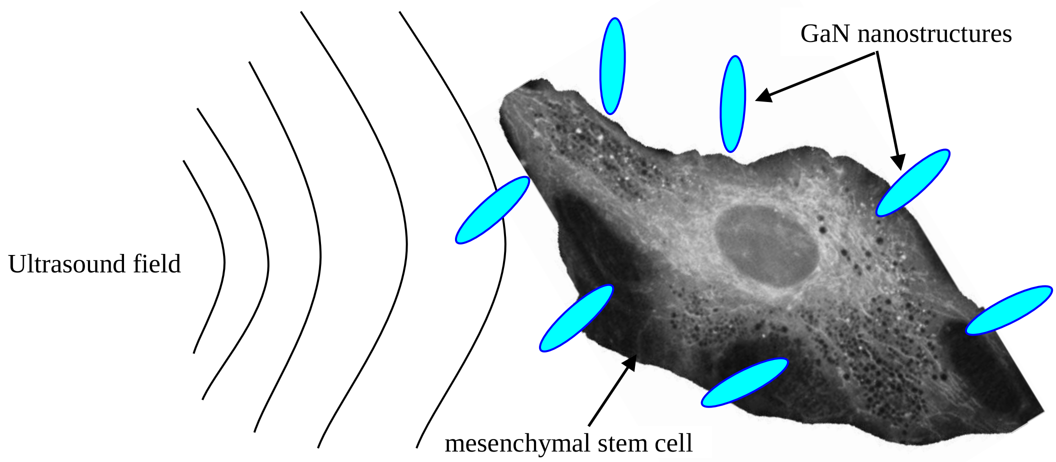

Fig.1. Schematic representation of remote electrical stimulation of cells using GaN nanostructures

II. RESULTS

GaN nanoparticles are fabricated using Surface Charge Lithography developed at the National Center for Materials Study and Testing, Technical University of Moldova [11]. This cost-effective technique allow us to obtain ultra thin membranes, different shapes micro- and nanostructures of GaN (fig. 2) using selective surface treatment of bulk material with low energy plasma or focused ion beam equipment and subsequent photoelectrochemical etching.

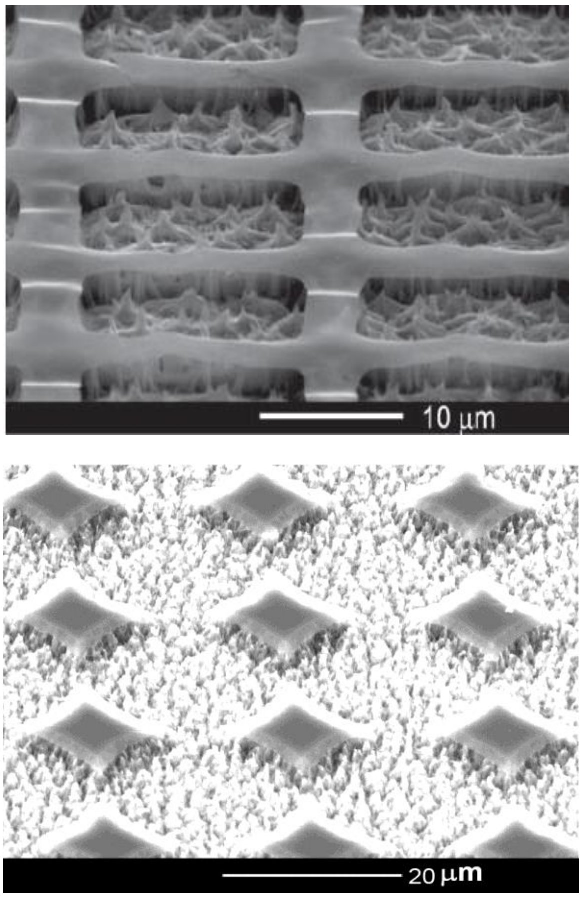

Fig.2. SEM images of different GaN nanostructures fabricated by SCL technique.

The obtained nanostructures were collected by ultrasonication in cultivation medium DMEM and added to cultivation medium. The mesenchymal stem cells were obtained from the skeletal tissue of the chicken fetus, after dissociation were growth in the CO_2 incubator at 37^0C, humidified atmosphere (95%) for 14 days. Embryos have been extracted from eggs and dissected to remove the femurs and tibias. Obtained bones have been mechanically processed in small fragments. The mass has been enzymatically processed with 0,25% Trypsin/EDTA (Sigma) and meanwhile mixed for 20 min with a magnetic stirrer. After of the enzymes inactivation with inactivation medium (DMEM+20%FBS, Sigma), the obtained suspension has been centrifuged at 300g for 10 min. The precipitate has been diluted with DMEM to receive a cellular suspension. Cells have been counted in the Goreeav chamber. The cells were planted in 96-well (NUNC), at the concentration of 15.000 cell/cm² in triplicate for each experimental group. There were four groups: I – without ultrasound; II – cells and nanostructures without ultrasound; III – cells under the action of ultrasound field; IV – cells and nanostructures under the action of ultrasound field.

The ultrasound field was generated using a piezotransducer connected to variable frequency generator. The piezotransducers has been attached to the culture dishes with ultrasound gel (Aquasonic). The stimulation was performed periodically for 15 minutes per hour during incubation period of 3 days. During the incubation period, the cells were analyzed for their morphology at the Inverted Phase Contrast Microscope (KXD), after three days of cultivation the adderent cells have been counted per each well.

Our preliminary results demonstrates that the number of cells have been increased by 23 % (± 2%) in cell cultures under the action of external ultrasound stimulation. Morphological analysis and viability shows no differences between the control group and the group studied.

III. CONCLUSION

GaN nanostructures have no toxic effect on nucleated cells, at least for a concentration up to 105 particles per □l. The cells proliferation enhancement up to 23 % (± 2%) shows that GaN nanostructures remotely stimulated by ultrasound field has an stimulating effect on cells proliferations. This parameter can be significantly improved in our oppinion by proper adjustment of stimulation parameters and the design of GaN nanostructures.

The proposed technique will be developed and could find applications for post-operative healing enhancement or for stimulation of tissue regeneration in delayed healing.

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