INSTITUTE OF MOLECULAR GENETICS
AND GENETIC ENGINEERING
University of Belgrade

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# Immobilized NT2/D1 Cells in Alginate Fibers: A Promising 3D Model System for Investigating Human Neurogenesis and Screening the Effect of Drugs and Bioactive Compounds

**Jelena Pejić**[1], Marija Mojsin[1], Jasmina Stojkovska[2], Aleksandra Medić[1], Isidora Petrović[1], Milena Stevanović [1,3,4], Bojana Obradović [2], Milena Milivojević [1]

*[1] University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, [2] University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia, [3] University of Belgrade, Faculty of Biology, Belgrade, Serbia, [4] Serbian Academy of Sciences and Arts, Belgrade, Serbia*

**Introduction**: The NT2/D1 embryonal carcinoma cell line represents a well-established *in vitro* model of human neurogenesis. It's widely used for studying neurodevelopmental processes, neurotoxicity, and neurodegenerative disorders. The utilization of alginate fibers as a 3D cell culture system offers a biocompatible and structurally supportive environment for neural differentiation and maturation of cells, making it a suitable tool for investigating neurodevelopmental processes.

**Methods**: In this study, we evaluated the alginate microfibers as a 3D model system for *in vitro* neural differentiation of NT2/D1 cells. We described the immobilization of NT2/D1 cells in alginate microfibers and the effect of propagation in this 3D model on morphological features, viability, and proliferation of immobilized cells. We also assessed the RA-induced initiation of neural differentiation of NT2/D1 cells in alginate microfibers by comparison with the initiation of neural differentiation in adherent 2D cell culture.

**Results:** Our results showed that immobilized NT2/D1 acquired morphological features characteristic of cells propagated in 3D model systems and retained viability, proliferative capacity, and ability to attach to adherent surfaces. In addition, immobilized NT2/D1 cells preserved neural differentiation capacity. Upon RA-induction, we detected a marked decrease in the expression of specific pluripotency-maintaining markers, *SOX2*, *OCT4,* and *NANOG*. Consecutively, the expression of early neural markers, *SOX3*, *PAX6,* and *miR219* was significantly increased.

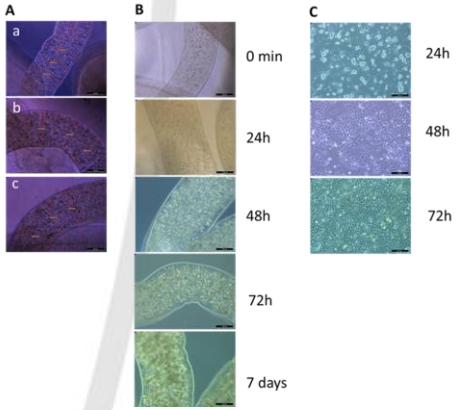


**Figure 1**: **Light miscroscopy of NT2/D1 cells immobilized in alginate fibers** (A) Aginate fibers diameter (µm) (B) NT2/D1 cells immobilized in alginate fibers for indicated periods of time (C) NT2/D1 cells released from alginate fibers and propagated in 2D culture for indicated periods of time. The scale bar is 100 µm.

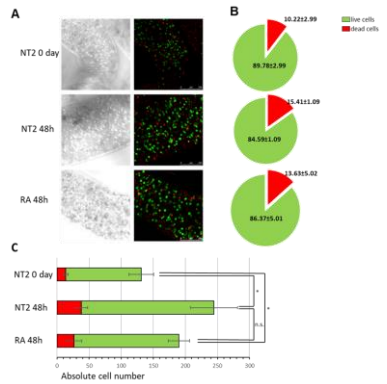


**Figure 2**: **Live/dead assay on untreated and RA-induced NT2/D1 cells immobilized in alginate fibers** (A) Bright-field and fluorescence confocal microscopy of live (green) and dead (red) NT2/D1 cells. (B) Pie charts showing the percentage ratio of live and dead NT2/D1 cells counted in cross-section images. (C) Column chart representation of absolute cell number and live/dead NT2/D1 cell ratio in cross-section images.

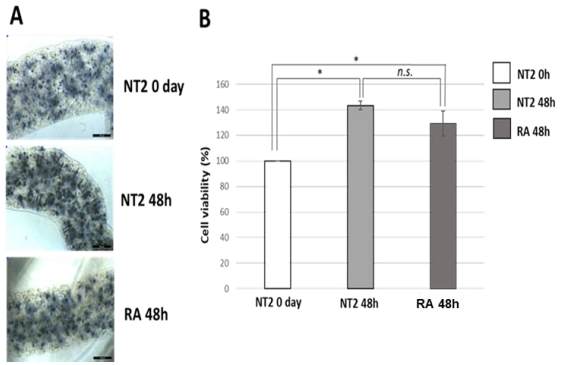


**Figure 3: MTT assay on untreated and RA-induced NT2/D1 cells immobilized in alginate fibers** (A) Light microscopy of formazan crystals formed within alginate fibers (B) Column chart representation of NT2/D1 cell viability.

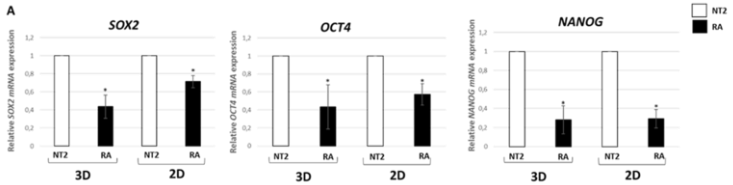


**Figure 4: Expression of pluripotency genes SOX2, OCT4, and NANOG during initiation of RA-induced neural differentiation of NT2/D1 cells immobilized in alginate microfibers**

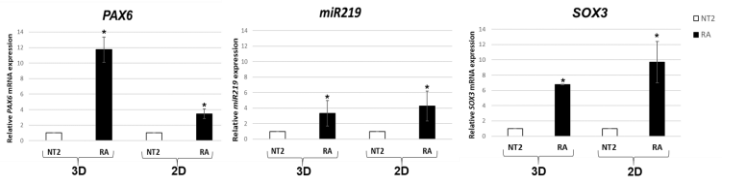


**Figure 5: Expression of early neural markers PAX6, miR219, and SOX3 in the initiation of RA-induced neural differentiation of NT2/D1 cells immobilized in alginate microfibers**

**Conclusion: Neural differentiation of NT2/D1 cells immobilized within alginate fibers represents a highly promising 3D model system for studying human neurogenesis and offers a valuable platform for screening the effect of drugs and bioactive compounds on human neural differentiation**