



Trends in **Molecular Biology** • Special issue

Abstract Book

CoMBoS²

2nd Congress of Molecular Biologist of Serbia

Belgrade • 2023

ISBN-978-86-82679-15-8



**CoMBoS2 – the Second Congress of Molecular Biologists of Serbia,
Abstract Book – Trends in Molecular Biology, Special issue**

06-08 October 2023, Belgrade, Serbia

Online Edition

<https://www.imgge.bg.ac.rs/lat/o-nama/kapacitet-i-oprema/istrazivacka-delatnost>

<https://indico.bio.bg.ac.rs/e/CoMBoS2>

IMPRESSUM

PUBLISHER:

**Institute of Molecular Genetics and Genetic Engineering (IMGGE),
University of Belgrade**

FOR THE PUBLISHER:

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Institute of Molecular Genetics and Genetic Engineering (IMGGE),

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Belgrade, 2023

ISBN 978-86-7078-173-3

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Belgrade • 2023

Content

Welcome speech 4

Congress Organizers 5

MolBioS Award Winner 9

Plenary speakers 10

Session plenary speakers

- MOLECULAR BIOMEDICINE 11
- MOLECULAR BIOTECHNOLOGY 13
- MOLECULAR MECHANISMS OF CELL FUNCTIONS 16

Abstracts

• Session PLENARY LECTURES 20

• Session MOLECULAR BIOMEDICINE 25

PLENARY LECTURES 26

INVITED LECTURES 31

POSTERS 38

Session MOLECULAR BIOTECHNOLOGY 100

PLENARY LECTURES 101

INVITED LECTURES 107

POSTERS 112

• Session MOLECULAR MECHANISMS OF CELL FUNCTIONS 126

PLENARY LECTURES 127

INVITED LECTURES 134

POSTERS 139

• MolBioS Student Session 157

Project Corner 182

Congress Friends 190

Sponsors 191

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

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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 bio-compatible 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 retain 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.

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.

Key words: NT2/D1 cell line; neural differentiation; alginate fibers; 3D model system

Acknowledgements: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no. 451-03-47/2023-01/ 200042) and European Union's Horizon Europe programme (Grant Agreement Number 101060201, STREAMLINE).