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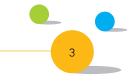
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Introduction: The NT2/D1 embryonal carcinoma cell line represents a well-established *in vitro* model of human neurogenesis. It's widely used for studying neurodevelopmental processes, neurotoxicity, and neurodegenerative disorders. The utilization of alginate fibers as a 3D cell culture system offers a 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 and ginate 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

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Abstracts