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| 論文題目 | Biomaterials for neural cells replacement therapy<br>（神経細胞の移植治療に用いる生体材料） | | |

（論文内容の要旨）


This thesis describes the design and preparation of polymeric biomaterials for the application in the neural cell replacement therapy. The thesis consists of a general introduction, five chapters, and summary of obtained results.

The General Introduction approaches the neural stem cells (NSCs) replacement therapy as one of the most promising technique for the treatment of neurodegenerative disosrders. Although the poor survival of transplanted cells and low cell integration with the host tissue still limit the the results of the therapy. The feasibility to develop polymeric biomaterials as a scaffold for neural stem cells transplantation to overcome the aforementioned limitations are discussed and proposed.

In Chapter 1, the preparation and study of a bi-functional His-tagged fusion protein that encompasses epidermal growth factor (EGF), a molecule known for inducing cell proliferation, combined with a collagen binding domain (CBD) derived from the von Willebrand Factor is reported. The designed fusion protein (EGF-CBD-His) could bind to the collagen substrate with its CBD end, presenting then the EGF to the NSCs. The designed system could maintain the initial cell seeding up to one week whereas only 20% of cell survived after 1week in the collagen without the EGF-CBD-His protein.

In the Chapter 2, a second bi-functional His-tagged fusion protein is reported. In this chapter the neural cell adhesion molecule (NCAM) was combined with a CBD derived from the decorin protein. The NCAM-CBD-His protein was designed to bind to the collagen fibers via its CBD end providing then a specific anchorage site for the NSCs. Although collagen is suitable for NSCs culture, it lacks specific NSCs adhesion molecules for appropriate cell adhesion. In this chapter it is demonstrated that NCAM-CBD-His improves the NSCs affinity to the collagen substrate without interfering on cell phenotype and improving the cell survival at 46%.

In the Chapter 3, another cell binding protein was fused with the CBD adopted in the Chapter 2. The neural cadherin (n-cadherin) is a calcium dependent cell adhesion protein. The n-cadherin-CBD-His protein also improved cell adhesion on collagen substrate, and improved neural stem cells differentiation to neuron cells. Cells cultured in the three-dimensional collagen hydrogels treated with n-cadherin-CBD-His present longer neurites and better cell network formation, which is closer to the native tissue environment.

In the Chapter 4, a DNA hybridization system to label NSCs for MRI monitoring post-transplantation is reported. The DNA hybridization system via DNA modified superparamagnetic iron oxide (SPIO) nanoparticles showed to be non-toxic for NSCs at concentrations up to 50 μg/mL. Labeled cells could form aggregates and presented similar phenotype as the non-labeled cells. Both labeled cell aggregates and single cells were detected by MRI in vitro as well in vivo. The DNA hybridization system for NSCs could

appropriately label cells in a specific and relatively fast two-step process.

In the Chapter 5, human iPS cells derived dopamine releasing neurons were differentiated in a long-term free-floating culture. Cells were expanded in a 96-well plate for 5 days and encapsulated within calcium-alginate microbeads hydrogels. Calcium-alginate hydrogels gelation is reversible with chelating agents such as citrate buffer. Aggregates released from the calcium alginate shell demonstrated to release dopamine at same level as the cells cultured in adherent culture (positive control). Transplanted cells into the rat brain striatum for 1 week survived and were positive for dopamine, demonstrating the feasibility of the proposed method for long-term differentiation of dopamine releasing neurons in calcium-alginate microbeads.

In summamry, the thesis describes the preparation of three injectable modified collagen hydrogels as a neural cells scaffold, a method to monitor cells post-transplantation, and a method to obtain in large scale mature neurons from human induced pluripotent stem cells.