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[P03-425] Phase Separation-driven Fabrication of Microparticle-connected Porous Hydrogel Scaffolds for 3D Cultivation of Liver Cells

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Keywords：3D cell culture、Porous scaffolds、Phase separation、Liver cell、Hydrogel

[Purpose]
3D culture techniques for mammalian cells are useful in a variety of biomedical applications, including regenerative medicine and cell-based drug evaluation. Various approaches using porous materials as scaffolds have been proposed, which were fabricated by electrospinning and freeze-drying. However, uniform cell seeding within porous scaffolds remains a challenge.In this study, we developed a new process to prepare unique, porous hydrogel scaffolds, which are composed of interconnected microparticles. A density-balanced aqueous two-phase system (ATPS) was utilized to form the porous hydrogel, and the pore connectivity and the particle size were controlled. The prepared scaffolds were applied to the 3D culture of liver cells and their functions were characterized.[Method]
An ATPS composed of gelatin methacrylate (GelMA) and dextran (Dex) was employed, in which the densities of the two phases were balanced. A photoinitiator was added to the mixture, followed by stirring and UV irradiation to selectively crosslink the GelMA-rich phase. Subsequently, the Dex-rich phase was removed by washing to form interconnected pores. The obtained hydrogels were sectioned and subjected to staining for structural characterization. Human hepatoblastoma cell line (HepG2) were then applied by dropping a cell suspension. After cultivation, cell viability and gene expression levels were evaluated.
[Results]
In the GelMA/Dex ATPS, the densities of the two phases were successfully tuned by adjusting the concentrations of the polymers and balanced at 20% GelMA and 18% Dex. The resulting hydrogel exhibited a unique structure consisting of interconnected particle-shaped GelMA hydrogels. The obtained hydrogel showed high fluid permeability, which contributed to the uniform cell seeding within the scaffolds simply by dropping a cell suspension. Cells proliferated in the interparticle space, forming small-sized aggregates. HepG2 cells cultured in this scaffold exhibited high cell viability even after 14 days of cultivation. RT–qPCR analysis showed the enhanced expression of liver-specific genes compared with conventional plate culture.
[Discussion]
The most important key to the successful creation of uniform scaffolds is the balancing of the densities of the two phases. Under optimized conditions, bicontinuous state of the two phases was achieved, which was stably maintained. As a result, the material exhibited higher interconnectivity of pores compared to conventional scaffolds. Also, cell viability and functions were improved relative to homogeneous hydrogel culture system used as a control, suggesting that the formation of interconnected micropores facilitated efficient transport of oxygen and nutrients to the cells.
[Conclusion]
In this study, we proposed a cell culture system using porous hydrogels fabricated by using ATPS and demonstrated its utility for 3D cell culture. Currently, we are working on optimizing the culture conditions to further enhance cellular functions.