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TATVHL peptide-grafted alginate/poly(γ-glutamic acid) scaffolds with inverted colloidal crystal topology for neuronal differentiation of iPS cells

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ABSTRACT

The neuronal differentiation of induced pluripotent stem (iPS) cells in scaffolding biomaterials is an emerging issue in nervous regeneration and repair. This study presents the production of neuron-lineage cells from iPS cells in inverted colloidal crystal (ICC) scaffolds comprising alginate, poly(γ-glutamic acid) (γ-PGA), and TATVHL peptide. The ability of iPS cells to differentiate toward neurons in the constructs was demonstrated by flow-cytometeric sorting and immunochemical staining. The results revealed that hexagonally arrayed microspheres molded alginate/γ-PGA hydrogel into ICC topology with adequate interconnected pores. An increase in the quantity of surface TATVHL peptide enhanced the atomic ratio of nitrogen and the adhesion efficiency of iPS cells in constructs. However, the effect of TATVHL peptide on the viability of iPS cells was insignificant. The adhesion and viability of iPS cells in ICC constructs was higher than those in freeform ones. TATVHL peptide raised the percentage of β III tubulin-identified cells differentiating from iPS cells, indicating that TATVHL peptide stimulated the neuronal development in alginate/γ-PGA ICC constructs. TATVHL peptide-grafted alginate/γ-PGA ICC scaffolds can be promising for establishing nerve tissue from iPS cells.

1. Introduction

Guided differentiation of induced pluripotent stem (iPS) cells in porous biomaterials is one of the most important topics in current biomedical development. The discovery of iPS cells was initiated by introduction of four genetic factors (Oct3/4, Sox2, c-Myc, and Klf4) into mouse embryonic fibroblasts [1]. Subsequent scientific evidences confirmed that several defined factors were also capable of reprogramming liver cells, stomach cells, adult neural stem cells, adult fibroblasts, keratinocytes, and adult adipose stem cells to iPS cells [2–6]. In addition, a number of biopolymers have been employed to differentiate and propagate iPS cells. For example, iPS cells were induced in silk scaffolds for osteogenic differentiation [7], in poly(L-lactide) nanofibers for tissue-engineered blood vessel [8], in poly(methyl vinyl ether-alt-maleic anhydride) for phenotypic proliferation [9], in graphene and/or graphene oxide for diverse cell lineages [10].

Inverted colloidal crystal (ICC) scaffolds were versatile matrices with de novo design on tightly controlled pore morphology for cell colonization [11]. In addition, adequate transparency of ICC hydrogels facilitated traditional optical instruments to scrutinize cell growth [12]. ICC topology also provided large surface area and high porosity for constant transfer of nutrients [13]. Moreover, homogeneous adhesion and migration of hepatocytes in ICC constructs enable a reproduction of multicellular spheroids [14]. In a study on differentiation of hematopoietic stem cells into B-lymphocytes, ICC scaffolds were used as an analog to imitate the function of bone marrow matrix [15]. Furthermore, ICC scaffolds with dermal fibers in their pores were verified to be of potential for sustaining skin integrity to connect percutaneous devices [16].

Alginate, composed of (1-4)-linked β-D-mannuronic acid (M-block) and α-L-guluronic acid (G-block), belongs to linear polysaccharide and is often used in nerve tissue engineering. For instance, neurosphere cells were imbedded in sodium alginate for ameliorating viability, migration, and differentiation and the cell-polymer constructs could be implanted into injured spinal cord to avoid a cell loss [17]. In addition, alginate-based anisotropic capillary hydrogel was found to be a proper substrate for adhesion of adult neural progenitor cells and axonal growth [18]. Moreover, poly(γ-glutamic acid) (γ-PGA) is a hydrophilic homopolymer with high antibacterial activity and low immunogenicity [19]. In a study on gene transfection, γ-PGA was hybridized with chitosan/DNA

Abbreviation: iPS, induced pluripotent stem; SSEA, stage-specific embryonic surface antigen; TAT, transactivator of transcription; TATVHL peptide, NH2-YGRKKRRQRRRDTLKERCLQVVRSLVK-COOH; VHL, von Hippel-Lindau; γ-PGA, poly(γ-glutamic acid).

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