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Design of culture substrates for large-scale expansion of neural stem cells

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ABSTRACT

Neural stem cells (NSCs) have been frequently used to investigate in vitro the molecular and cellular mechanisms underlying the development of the central nervous system (CNS). In addition, NSCs are regarded as one of the potential sources for the cell replacement therapy of CNS disorders. Most of these studies have utilized NSCs prepared by neurosphere culture. However, this method normally yields a heterogeneous population containing differentiated neural cells as well as NSCs. In addition, the rate of cell expansion is not high enough for obtaining a large quantity of NSCs in a short period. Here we report the design of culture substrates that allow highly selective and rapid expansion of NSCs. We synthesize epidermal growth factor fused with a hexahistidine sequence (EGF-His) and a polystyrene-binding peptide (EGF-PSt), and these engineered growth factors were surface-anchored to a nickel-chelated glass plate and a polystyrene dish, respectively. The EGF-His-chelated glass substrate was further used to assemble a culture module. Neurosphere-forming cells prepared from the fetal rat striatum were used to examine the selective expansion of NSCs using the EGF-His-chelated module and the EGF-PSt-bound polystyrene dish. Our results show that the culture module enables to selectively expand NSCs in a closed system more efficiently than the standard neurosphere culture. The EGF-PSt-bound polystyrene dish also permits efficient expansion of NSCs, providing a straightforward means to acquire a large quantity of pure NSCs in standard laboratories.

1. Introduction

Neural stem cells (NSCs), capable of self-renewal and differentiation into multiple cell types, are found in embryonic and adult tissues in the central nervous system (CNS) [1]. During development, NSCs generate neurons, astrocytes, and oligodendrocytes to organize nervous tissues and are considered to contribute to the neurogenesis in the adult CNS [2,3]. To date, numerous studies have been made with NSCs cultured in vitro to investigate the molecular and cellular mechanisms underlying mammalian CNS development [4,5]. In addition, NSCs have been utilized as one of the potential sources for the cell replacement therapy of CNS disorders [6,7].

All of these fundamental and applied studies largely rely on the capability of culturing NSCs in vitro. Currently, the most standard method to obtain NSCs is so-called neurosphere culture [8,9], in which neural cells dissociated from embryonic or adult tissues are cultured in suspension in the presence of basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). As a result, growth factor-responsive cells proliferate to generate cell aggregates, neurospheres, in which NSCs are enriched.

Though the neurosphere culture is widely employed, there are still limitations associated with this method. The most critical problems may be heterogeneity of cell populations in a neurosphere. The content of cells expressing a marker for NSCs, nestin, reaches only 50–60% of total cells in a neurosphere [10]. The rest of cells are more differentiated cells in neuronal and glial lineages. Such heterogeneity makes it difficult to solely investigate the behaviors of NSCs, and also limits the controlled processing of NSCs for cell transplantation therapy. In addition, a growth rate is limited in neurosphere culture, probably because of the fact that differentiated cells present in neurospheres have reduced potential of proliferation [11]. Accordingly, it appears that the neurosphere culture method is inefficient for the production of NSCs.

To overcome these limitations, we previously developed a culture substrate that enables highly efficient expansion of NSCs in adherent culture [10,12]. When neurosphere-forming cells prepared from the fetal rat striatum were cultured on the substrate, a population rich in NSCs (> 98%) could be obtained more rapidly (32-fold) than in neurosphere culture. On the substrate, EGF, a strong mitogen for NSCs [8], was immobilized for selectively capturing NSCs and transducing proliferative signaling in the captured NSCs. To avoid denaturation of EGF upon immobilization and detachment of immobilized EGF during cell culture, a hexahistidine sequence (His) was fused to the C-terminus of EGF [13]

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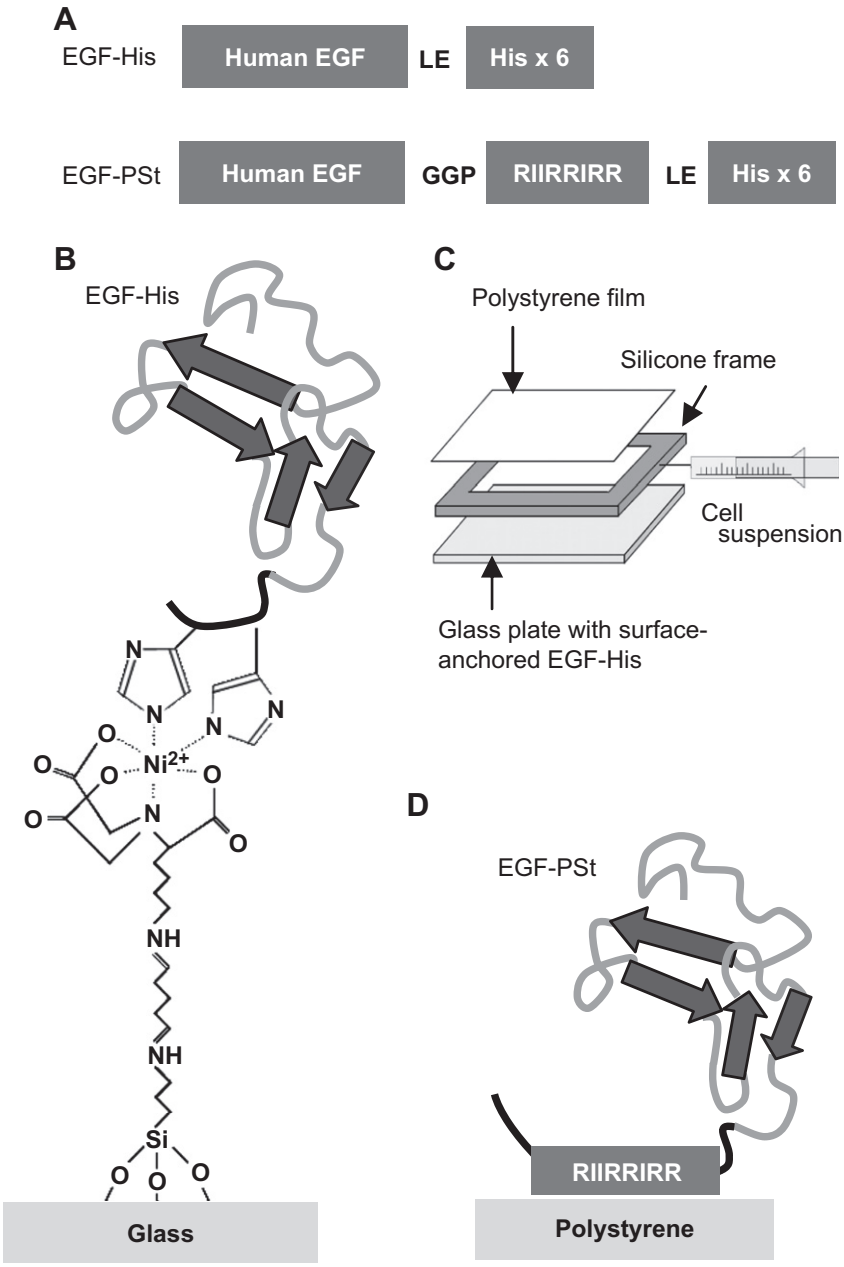

Scheme 1. (A) Structure of EGF-His and EGF-PSt. The sequence LE comes from the plasmid. The sequence GGP was inserted as a linker. His: hexahistidine. (B) Illustration for the oriented immobilization of EGF-His onto a glass surface. (C) Illustration of a culture module fabricated using a glass plate with surface-anchored EGF-His. (D) Illustration for the surface anchoring of EGF-PSt onto a polystyrene surface.

and then the engineered EGF (EGF-His) was anchored under mild conditions through the coordination of His with a Ni^{2+}-bearing alkanethiol monolayer formed on a gold-evaporated glass plate.

The method for surface-anchoring EGF opened a way to fabricate culturewares that allow for large-scale expansion of pure NSCs. The present study was undertaken to construct culture modules with surface areas much larger than the laboratory-scale substrate that we previously reported [10,12]. For uniformly anchoring EGF-His over a larger area, attempts were made to utilize a glass plate with amine functionalities at the surface. It also seems that no requirement for a gold layer is advantageous to the general use of the technique. In the present study, the surface-anchoring method was further applied to conventional polystyrene dishes. For simple fabrication, a nonapeptide (RIIIRRIRR) bearing an affinity for polystyrene [14] (designated this peptide as PSt tag hereafter) was fused to EGF. The EGF-PSt was then anchored to the surface of a polystyrene dish by simply exposing the dish to the EGF-PSt solution.

2. Materials and methods

2.1. Plasmid construction

The preparation of an expression vector for EGF-His (pET22-EGF) was described elsewhere [13]. For the construction of plasmid for EGF-PSt, a coding sequence for EGF was amplified from pET22-EGF by polymerase chain reaction (PCR) using a sense primer, 5′-agatatacatatgaatagtgactctgaatgtcccctg-3′, and an antisense primer, 5′-ggtgctcgagTCGTCGGATCCTTCGGATGATGATACGtggacctccgcgcagttcccaccacttcag-3′ (both synthesized by Invitrogen, Carlsbad, CA). The antisense primer contained a sequence for PSt tag (uppercase letter) [14] and a linker peptide GGP (underlined). PCR was performed under the following cycling conditions: initial denaturation for 5 min at 94 °C; 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 30 s at 72 °C; final extension for 7 min at 72 °C. The DNA fragment thus