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A neural stem/precursor cell monolayer for neural tissue engineering

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ABSTRACT

The purpose of this study was to prepare a monolayer of neural stem/precursor cells (NSPCs) for neural tissue engineering applications. Two components present in serum, fibronectin and epidermal growth factor (EGF) were added into DMEM/F12 medium (termed medium B) to examine the effect of the migration-, proliferation- and differentiation-promoting potential on the cultured NSPCs, isolated from embryonic rat cerebral cortex. Compared with the serum effect, medium B also permitted neurosphere attachment onto the substrate surface and cell migration out of neurospheres extensively, but enhanced more extensive cell division and slowed down NSPC differentiation to generate a confluent NSPC monolayer. It was found the medium B-treated NSPCs possessed the capability to form typical neurospheres or to undergo differentiation into neuron-related cell types on various biomaterial surfaces. Therefore, we proposed a two-stage process for wound healing or nerve conduit preparation. Extensive NSPC division and MAP2-positive neuron differentiation were manipulated in NSPCs cultured in the medium B followed by the neuronal differentiation-favorable medium. These results should be useful for controlling the proliferation and differentiation of NSPCs on various biomaterials and conduits in neuroscience research.

1. Introduction

Neural stem/precursor cells (NSPCs) not only possess the ability to self-renew but also have the potential to differentiate into various cell types in vitro [1,2]. Many investigators have concentrated on elucidating the role of extrinsic and intrinsic signals coming from microenvironmental cues in the regulation of proliferation and differentiation of NSPCs [3–5]. For example, NSPCs can be expanded in the presence of mitogens, such as epidermal growth factor (EGF) or basic fibroblast growth factor (bFGF), to form free-floating neurospheres [6–8]. Under appropriate conditions, these neurospheres can attach onto substrates and can be induced to differentiate into neurons, astrocytes, and oligodendrocytes [9,10]. Furthermore, several in vitro systems have been described for increasing neurosphere formation and neuronal differentiation of NSPCs by using various growth factor combinations [11–14]. These protocols have been widely used to generate a variety of differentiated neural cell types for a wide range of clinical applications that may in the future lead to therapeutic approaches for treating neuronal disease.

Serum, a complex mixture containing a variety of components, plays an important role in supporting the survival or the proliferation of numerous types of cultured cells. It has been shown that floating neurospheres attach onto the substrate surface and NSPCs are principally induced to differentiate into astrocytes when the medium contained 10% fetal bovine serum (FBS) [10,15,16]. Removing the higher molecular weight components from FBS, our previous study demonstrated that molecules promoting neuron differentiation were present in serum with molecular weight <100 kDa, which could dominate the differentiation of NSPC principally into neurons [17]. Based on our previous results, we propose that serum fraction with molecular weight $>$ 100 kDa can guide NSPCs to exhibit different proliferation or differentiation behaviors. For simplifying the complicated composition of serum fraction, fibronectin and EGF, two components present in serum, were selected to add into the culture medium to investigate their effects on NSPC behaviors. Up to now, culture media have never been systematically identified to maintain NSPCs with nestin-positive phenotype when they attach onto the substrate surface. Therefore, the purpose of this study was to investigate whether EGF might cooperate with fibronectin in the medium to guide NSPC adhesion and proliferation for various neural tissue engineering applications.

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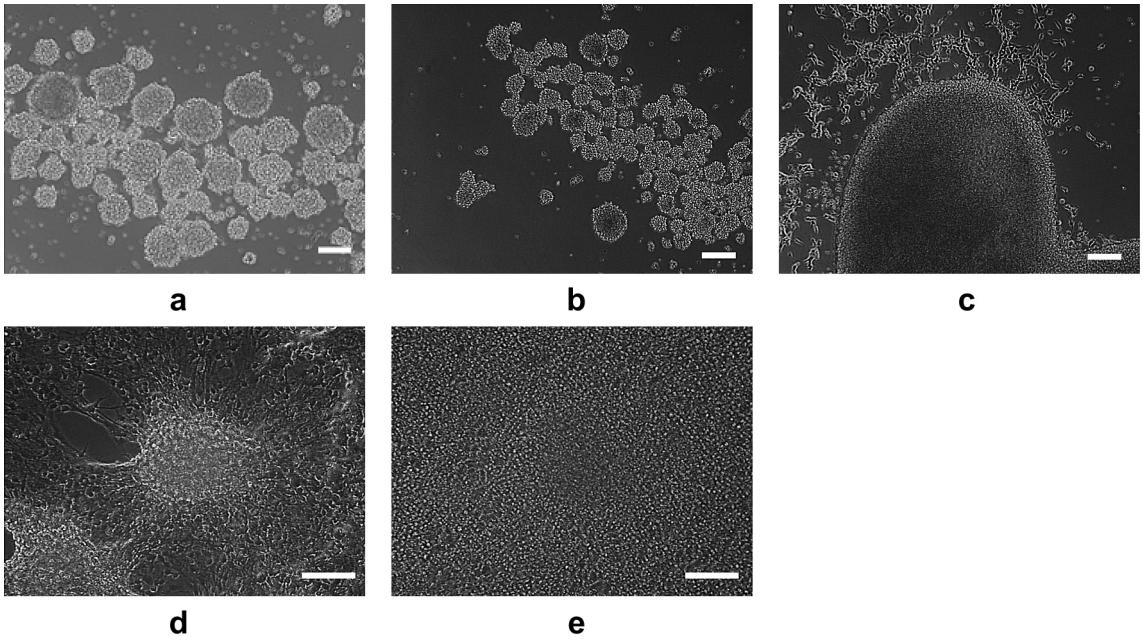

Fig. 1. Phase-contrast images of neurospheres cultured on TCPS in (a) DMEM/F12 medium, containing (b) 1 μg/ml fibronectin, (c) 10 ng/ml EGF, (d) 10% FBS, and (e) medium B after 7 days of incubation. Scale bar = 100 μm.

2. Materials and methods

2.1. Isolation and culture of NSPCs

NSPCs were obtained from pregnant Wistar rat embryos on day 14–15 according to a protocol detailed previously [18,19]. Briefly, rat embryonic cerebral cortices were dissected, cut into small pieces and mechanically triturated in cold Hank's balanced salt solution (0.4 g/l KCl, 0.09 g/l $Na_2HPO_4 \cdot 7H_2O$, 0.06 g/l KH_2PO_4, 0.35 g/l $NaHCO_3$, 0.14 g/l $CaCl_2$, 0.10 g/l $MgCl_2 \cdot 6H_2O$, 0.10 g/l $MgSO_4 \cdot 7H_2O$, 8.0 g/l NaCl, and 1.0 g/l D-glucose in deionized water). The dissociated cells were collected by centrifugation and were resuspended in the DMEM/F12 medium containing

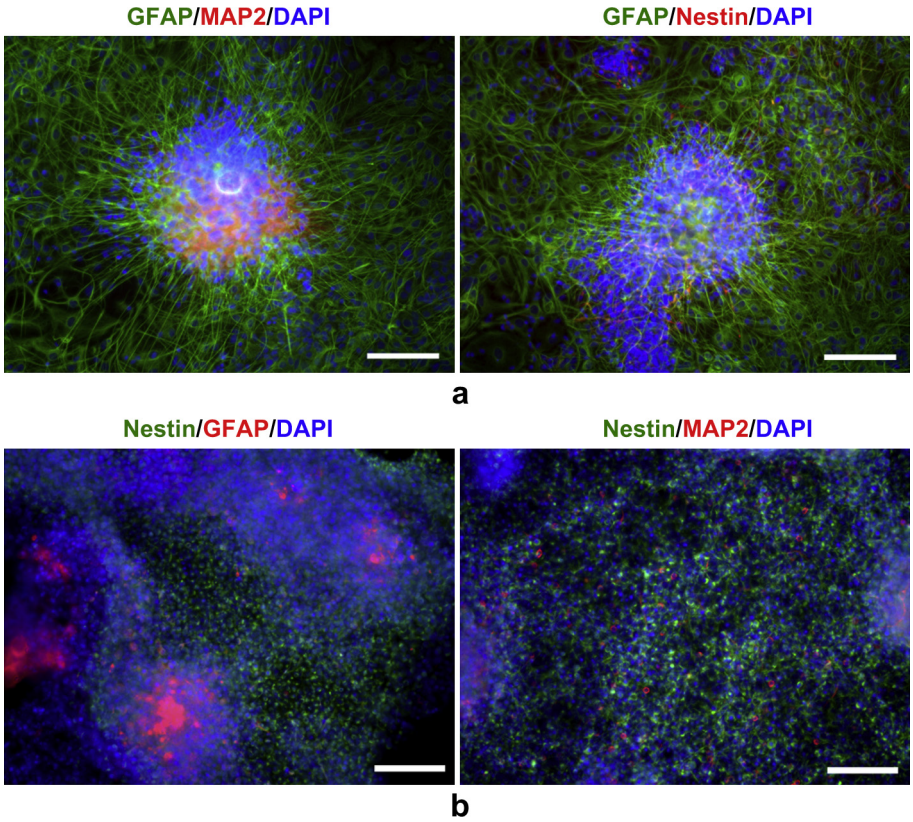

Fig. 2. Fluorescent images of neurospheres cultured on TCPS in the (a) 10% FBS-containing medium and (b) medium B after 7 days of incubation. Anti-Nestin, anti-MAP2 and anti-GFAP are immunoreactive for undifferentiated NSPCs, differentiated neurons and astrocytes, respectively. DAPI (blue) was used to mark nuclei. Scale bar = 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)