Control of Surface Density and Distribution of FGF2 for Neural Stem Cell Expansion

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Statement of Purpose:

Neural stem cells are currently being investigated for their potential application in treating neural degenerative diseases. An efficient expansion method is crucial to generating sufficient number of NSCs for clinical applications. The objective of this study is to design and synthesize a basic fibroblast growth factor (FGF2)-functionalized poly (ethylene terephthalate) (PET) substrate with well-controlled FGF2 density and distribution, and to investigate the effect of FGF2 density and distribution on NSC expansion and phenotype maintenance.

Methods:

PET film was biotinylated by aminolysis with ethylenediamine following by reacting with *N*-hydroxysuccinimidobiotin (NHS-biotin, Pierce). Biotinylated FGF2 (bFGF2) was prepared by reacting FGF2 with NHS-PEO-biotin (Pierce) at a molar ratio of 1:30. Streptavidin was used as a linker between biotinylated PET (bPET) and bFGF2. Various amount of bFGF2 was conjugated to bPET substrates with different surface-biotin densities. Adult rat neural stem/progenitor cells (green fluorescence protein transfected cells mixed with wild type at a ratio of 1:20) were cultured on these FGF2-immobilized PET substrates. Total cell *vs.* nestin$^+$ cell expansion rates, self-renewal and differentiation, and p42/p44 signaling activation were characterized by GFP colony size, immuno-staining for progenitor cells (nestin$^+$) and oligodendrocytes (RIP$^+$), and Western blotting analysis, respectively.

Results and Discussion:

Due to the highly specific binding between biotin and streptavidin, about 200 ng/cm^2 bFGF2 can be conjugated onto bPET substrate at efficiency higher than 60%. Varying biotin concentration during biotinylation was the best method to control biotin density on PET surface. The highest biotin density on bPET surface obtained in this study was 17 biotin molecules/100 nm^2. Streptavidin-HRP was used as a marker to examine the efficiency of protein conjugation on bPET surface; it is interesting to note that the maximum conjugation efficiency was not achieved on bPET with the highest biotin density. This is likely due to crosslinking of streptavidin with high density of surface biotin. A “clustering” FGF2 presentation can be achieved on surface with a lower biotin density and a medium FGF2 concentration, in contrast to “singular” FGF2 presentation obtained with higher biotin and high FGF2 densities (Fig. 1).

NSCs culture showed that FGF2 distribution is a crucial cue in determining the efficiency of NSC expansion (proliferation and phenotype maintenance). NSCs on PET substrate with clustering FGF2 presentation proliferated

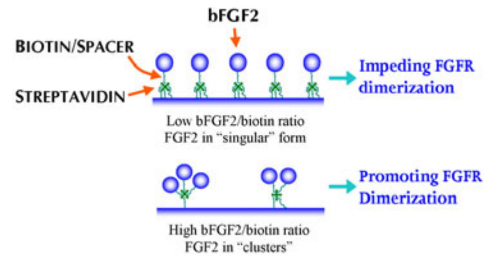

Fig. 1. Schematic diagrams of different FGF2 distribution on PET surfaces at same surface density.

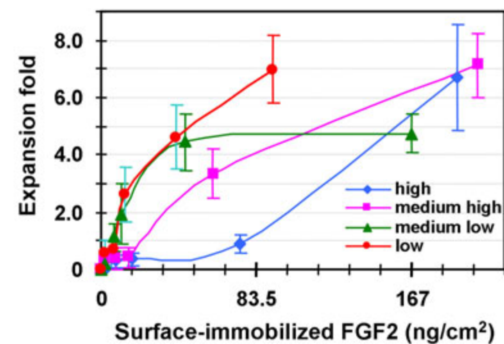

Fig. 2. Nestin+ clonal size on FGF2 conjugated PETs with different biotin density for 6 days.

significantly faster than cells on singular FGF2 surface (Fig. 2). Immunostaining showed higher RIP$^+$ staining among cells cultured on singular FGF2 substrate. The Erk1/2 pathway is a well-characterized MAP kinase signaling pathway and thought to be critical for NSC self-renewal[1]. Western blotting for phosphorylated p42/p44 MAP kinases indicated that the pErk1/2 level was significantly higher in NSCs cultured on PET with “clustering” FGF2 presentation in contrast to that on surface with “singular” form FGF2.

Conclusions:

Surface immobilization of FGF2 on PET substrate is achieved via a streptavidin-biotin linkage. Surface density and spatial distribution of FGF2 on PET substrate can be modulated by the control of biotin density on PET surface. Our analysis demonstrates the significance of spatial distribution of FGF2 in affecting NSC proliferation and phenotype maintenance.

References:

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