Biological tethering of N-Cadherin to Substrates Promotes Cell-Cell Adhesion

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Statement of Purpose: N-Cadherin-mediated cell-cell adhesion plays an important role in diverse cellular functions. However, it is a still challenge to understand the effects of cell-cell adhesion in vitro because of difficulty in regulating the extent and numbers of cell-cell contacts. Here, we hypothesize that biochemical coupling of cadherin molecules to protein linkers using a Fc-antibody would be more advantageous to stimulating cell-cell adhesion to target substrates, and further modulating cellular adhesion morphology and differentiation. We examined this hypothesis by chemically coupling an Fc-antibody to a substrate using a polyaspartamide protein linker and subsequently exposing them to controlled number of Fc-tagged N-cadherin (N-Cad-Fc). We analyzed the beneficial role of biological coupling of N-cadherin in stimulating cellular adhesion and also neural differentiation, as compared to chemical coupling of N-cadherin to the substrates. We believe that this conjugation method will be useful to coupling other types of cadherin (e.g., epithelial-cadherin, vascular endothelial- cadherin, etc) to various matrices and also controlling cellular phenotypic activities in a more elaborate manner.

Methods: First, the polysuccinimide (PSI) with the average molecular weight (M_w) of 57,000 g/mol was synthesized by acid-catalyzed polycondensation of L-aspartic acid. Then, designated amounts of 2-aminoethyl methacrylate, ethanolamine, and excess amounts of ethylenediamine were added sequentially to PSI dissolved in dimethylformamide, in order to prepare poly(2-amino-2-hydroxyethyl-co-2-methacryloxyethyl aspartamide) (PAHMAA). Next, the PHMAA was modified to present controlled number of N-hydroxyl succinimidy (NHS) ester groups. The poly(ethylene glycol) disuccinimidyl ester (Mw 7,000 g/mol) was added into water dissolved with PHMAA, and the mixture was stirred overnight. The resulting PHMAA-g-PEGNHS was extensively dialyzed against distilled water, followed by lyophilization.

Anti-Fc-antibody (Sigma) was dissolved in PBS at 250 μg/mL. PHMAA-g-PEGNHS (5 mg) was added to 0.1 mL of each Fc-antibody solution, and stirred at 4 °C overnight. The resulting products were mixed with 100 μL pre-gel solution (8 wt% acrylamide and 0.48 wt% N,N,N',N'-methylenebis(acrylamide) (bis-acrylamide)). Then, 10 μL of 10% ammonium persulfate (APS) and 5 μL of tetramethylethylenediamine (TEMED) were added to initiate the polymerization and cross-linking reaction. The pre-gel solution was then placed on a glass coverslip that was pretreated with 0.4% 3-(trimethoxysilylpropyl)methacrylate and immediately covered with an untreated cover slide. After incubation for 30 min, the coverslip was removed from the gel. Finally, the gel was incubated overnight at 4 °C with N-Cad-Fc in HEPES buffer at concentrations ranging from 0 to 100 and 250 μg/mL.

Results: We examined whether the N-cad can also modulate modulates adhesion of cortical neuron cells to a glass substrate and further neurite outgrowth. The sequential surface treatment of the glass with methacryloxypropyltrimethosylinane and mixture of Fc-antibody and PHMAA-g-PEGNHS resulted in a glass substrate that immobilized the Fc-antibody. The subsequent exposure of modified glass substrate to N-Cad-Fc led to the N-Cad-Fc conjugated glass substrate. Separately, chemical reaction between N-Cad-Fc and PHMAA-g-PEGNHS on the silane-treated glass also resulted in immobilization of N-Cad-Fc to the glass.

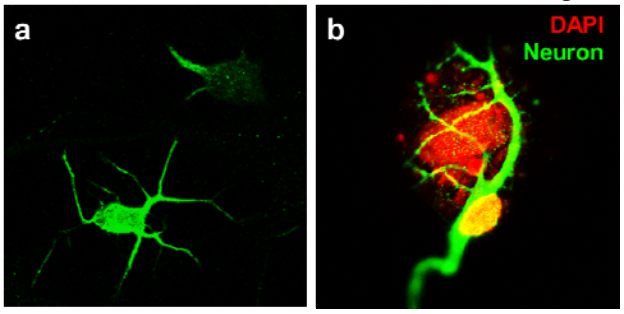

Figure 1. Confocal images of neural network formed on the substrate bound with N-Cad-Fc either by (a) chemical conjugation or (b) biological conjugation using Fc-antibody.

According to the confocal images, the substrate biochemically conjugated with N-Cad-Fc stimulated neurite outgrowth more significantly than the substrate chemically conjugated with N-Cad-Fc (Figure 1).

Conclusions: This work demonstrates a new method to tether recombinant N-Cad-Fc to substrates to regulate cell adhesion and neural network formation, by changing the surface density of N-Cad-Fc. In addition, biochemical conjugation resulted in more potent N-Cad-Fc activity than chemical attachment to the surface. The resulting system enabled us to modulate cell adhesion to the substrate surface by varying the N-Cad-Fc surface density. We believe that the biological tethering of N-Cadherin would be useful to modifying various biomaterial systems used for tissue regeneration and cell therapies.

References: *Biomacromolecules* **2014**, 15, 2172-2179.