Nanoscale Dendritic Clusters of RGD Peptides

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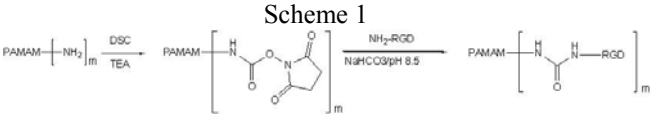
Statement of Purpose: Spatial distribution and aggregation of RGD peptides at the nanoscale significantly affect cell responses. Nanoscale clustering of RGD peptides can induce integrins to cluster. The clustering of integrins in the cell membrane helps to elicit of integrin-mediated cellular responses, and complete signaling only occurs when integrins are clustered in the cell membrane. Dendrimers are highly branched, nearly spherical and symmetrical, nanoscale macromolecules with high functionality, making them an ideal scaffold to construct nanoscale RGD cluster. In this study, synthetic strategies for the synthesis of RGD-dendrimer conjugates were explored based on anionic G3.5 and cationic G4.0 polyamidoamine (PAMAM) dendrimers. In addition, the RGD-PAMAM conjugates were evaluated in terms of cytotoxicity and cell adhesion inhibition and compared to unmodified PAMAM dendrimers and free RGD peptide.

Methods: The synthesis of RGD-PAMAM (G3.5) and RGD-PAMAM (G4.0) followed Scheme 1 and 2, respectively.

Scheme 1



Scheme 2

In order to test the cytotoxicity of peptide-loaded PAMAM dendrimers, triglycine (GGG) having similar structure to RGD was conjugated to G3.5 and G4.0 PAMAM dendrimers by following the Scheme 1 and 2, respectively. The cytotoxicity of parent dendrimers G3.5, G4.0 and GGG-conjugated G3.5 and G4.0 dendrimers to fibroblasts were evaluated *in vitro*.

The interaction between G4.0 PAMAM dendrimers with fibroblasts was visualized by coupling FITC to the dendrimer surface. G4.0 PAMAM dendrimers were labeled with FITC prior to measurement. Fibroblasts were seeded on the poly-D-lysine coated glass bottom culture dishes at 10^4 cells/mL. After 2 days, the medium containing FITC-G4.0 PAMAM at 0.422 mg/mL were used to replace the original medium for 15 min, 1 h, and 2 h, respectively. The cells were imaged with a Radiance 2100-Multi-Photon/Confocal Imaging system.

Dendritic peptide inhibition was tested for validating the functionality of the conjugated RGD peptides. Each 100 μL of freshly isolated fibroblasts at 10^4 cells/mL was seeded into the wells of a TCPS 96-well plate at the presence of 0, 0.77, 77 or 770 μM of free RGD, 64RGD-G3.5, or 64RGD-G4.0 at 37 °C in an atmosphere with 5% CO2 and 95% humidity. The medium used in this study was serum free and without any supplements. After 2 h, each well was washed by medium three times to remove unattached fibroblasts. Adherent fibroblast density was quantified based on measurements from three randomly selected fields using an Olympus TE300 phase-contrast microscope. In order to consider the effect of the cytotoxicity of dendritic scaffolds on cell adhesion, cell viability at 2, 24, and 96 h after 2 h-treatment with dendritic peptides was measured.

Results / Discussion: The peptide-PAMAM conjugates were characterized and confirmed by ^{1}H-NMR and GPC.

G3.5 PAMAM dendrimer did not induce a cytotoxic response in fibroblasts at 0.2, 2, or 20 μM for up to 96 h. The cytotoxicity of G4.0 PAMAM dendrimer was significantly reduced through the modification with GGG. However, the cytotoxicity reduction of G4.0 depends on the peptide loading degree on the dendrimer surface and the toxicity tolerance of fibroblasts to peptide-loaded G4.0 is time and concentration dependent. Meanwhile, cell morphology was consistent with cell viability changes. The cells without cytotoxic effect spread over; however, the cells with cytotoxic effect became rounded and started to die. At 20 μM, 64GGG-G4.0 has much less cytotoxic effect on cell morphology than 16GGG-G4.0 and 32GGG-G4.0 up to 24 h.

The confocal images have shown that FITC labeled G4.0 could enter inside fibroblast within 15 min. The strong fluorescence signals were found throughout the cytoplasm, indicating the major accumulation place of FITC labeled G4.0 PAMAM dendrimers inside fibroblast.

At 0.77 μM, the presence of free peptides had little effect on cell adhesion. So did RGD-3.5 and RGD-G4.0 at 0.77 μM. But, free RGD peptides decreased the number of adherent fibroblasts as the concentration increased. At 770 μM, RGD significantly inhibited cell adhesion. The cell density, after treatment with RGD at 770 μM for 2 h, dropped 70-75% compared to the control. We found the similar decrease in cell density as the concentration of dendritic peptides increased. RGD-G4.0 at 770 μM is most toxic, causing permanent damage to fibroblasts at 24 and 96 h, which, however, is not obvious at 2 h. The cytotoxicity of RGD-G4.0 at 77 μM is mild, causing 20% drop of cell viability. Therefore, the decrease of the adherence cell density at the presence of RGD-G4.0 was mainly due to its cytotoxicity. In contrast, RGD-G3.5 is not toxic as free RGD, and has shown comparable cell inhibition capability as compared to free RGD.

Conclusions: Anionic G3.5-based dendritic RGD in suspension has shown no negative effect on fibroblast viability and a concentration-dependent effect on lowering cell adhesion on TCPS as that of free RGD. Similar concentration dependent effect in cell viability and adhesion was observed for cationic G4.0-based RGD at lower but not at high concentrations.

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