Synthesis and Characterization of di-Functional PEG-based Crosslinkers for L1 Immobilization

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Statement of Purpose: L1 neural cell adhesion molecule (CAM) is a transmembrane, cell-cell adhesion molecule expressed in the developing nervous system that mediates axonal guidance and fasciculation. Recombinant L1 has been shown to support selective neuronal adhesion[1], neurite outgrowth[2], and neuronal survival[3]. While short peptide sequences have been discovered that provide the integrin binding function of many ECM adhesive proteins, all six immunoglobulin domains of L1 have been shown to be required for full bioactivity.[4] Based on the increased number and distribution of bioactive regions in L1, selection of an appropriate crosslinking chemistry is likely to be critical for immobilization with full retention of bioactivity. The objective of this study was the synthesis and characterization of di-functional crosslinkers targeting different amino acids and domains of L1 for immobilization to acrylic-based hydrogels and polymers.

Methods:

A. Synthesis of NTA-PEG-Acrylate (Figure 1): ε-amino-nitrilotriacetic acid (NTA) was synthesized following the procedure of Ho *et al*.[5] and NHS-PEG-Acrylate (MW=3400) was obtained from Nektar (Birmingham, AL). To synthesize NTA-PEG-Acrylate, NHS-PEG-Acrylate (200 mg) was added to a solution of ε-amino-NTA (100 mg) in 30 ml DMF while stirring at 50 ºC and allowed to react for 3 days at 50 ºC at dark. The molar ratio of NHS-PEG-Acrylate to NTA was 1:43. After 3 days, the reaction solution was filtered to remove unreacted NTA and DMF was removed by evaporation. The remaining residue was precipitated in cold ether to obtain NTA-PEG-Acrylate. Following purification, the structure of NTA-PEG-Acrylate was characterized by ^{1}H-NMR (D_2O).

B. Synthesis of PDEA-PEG-Acrylate (Figure 1):
2-(2-pyridyldithio)ethylamine hydrochloride(PDEA HCl) was prepared as described by Li *et al*.[6] and 28.3 mg (120 µmole) PDEA HCl was treated with TEA (1: 1.2 mole ratio) in 2 ml DMF to obtain 2-(2-pyridyldithio) ethylamine (PDEA). To synthesize PDEA-PEG-Acrylate, 200 mg (58.5 µmole) NHS-PEG-Acrylate(MW=3400) was dissolved in 3 ml DMF and added into above PDEA solution and allowed to react for 24 hours at room temperature. The solution was neutralized and dialyzed against water for 24 hours and recovered by lyophilization. The structure of PDEA-PEG-Acrylate was characterized by ^{1}H-NMR (D_2O).

Results/Discussion:

Two di-functional crosslinkers, NTA-PEG-Acrylate and PDEA-PEG-Acrylate were synthesized and characterized by ^{1}H-NMR (D_2O). Both reactions proceeded with high efficiency and conversion.

NTA-PEG-Acrylate: δ=3.45~3.90 (m, PEG backbone -CH_2), δ=4.24 (t, 2H, PEG acrylate terminal CH_2), δ=2.45 (t, 2H, PEG NTA terminal -CH_2), δ=3.4 (t, 2H, NTA epsilon -CH_2), δ=5.9 and 6.4 (d, 2H, acrylic –CH_2), δ=6.15 (q, 1H, acrylic -CH)

PDEA-PEG-Acrylate: δ=3.45~3.90 (m, PEG backbone -CH_2), δ=4.24 (t, 2H, PEG acrylate terminal CH_2), δ=2.45 (t, 2H, PEG PDEA terminal -CH_2), δ=5.9 and 6.4 (d, 2H, acrylic –CH_2), δ=6.15 (q, 1H, acrylic -CH), δ=7.2 and 8.3 (d, 2H, pyridyl -CH), δ=7.75 (t, 2H, Pyridyl -CH).

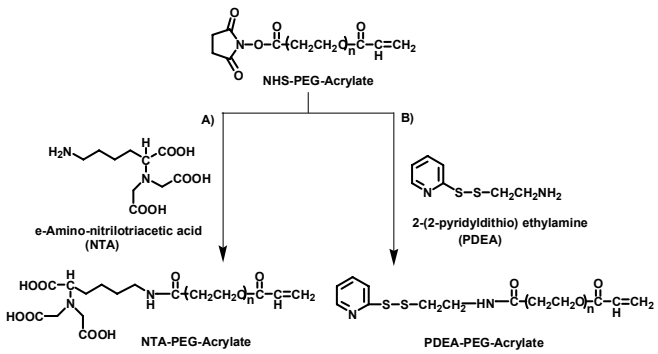

Figure 1. Synthesis route of NTA-PEG-Acrylate and PDEA-PEG-Acrylate

Conclusions:

We have previously described the cloning and expression of a soluble, 140 KDa physiological cleavage fragment of the L1 extracellular domain with a C-terminal 6X histidine tag and demonstrated full bioactivity.[7] On-going studies are evaluating immobilized L1 bioactivity for promoting neurite outgrowth based on oriented immobilization through the C-terminal His tag (NTA) and immobilization through lysine amine (NHS) and cysteine thiol (PDEA) amino acids (Figure 2).

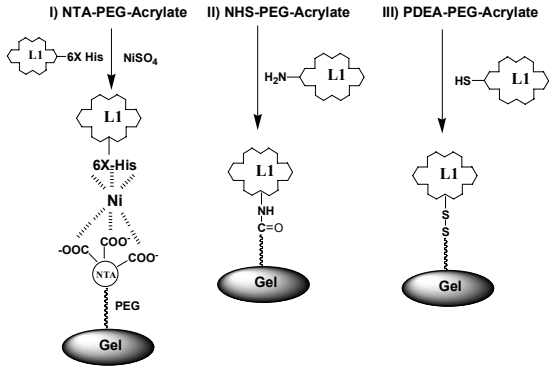

Figure 2. Proposed mechanisms of L1 immobilization using various di-functional cross-linkers

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