Glycosaminoglycans-mimicking polymers conjugated gold nanoparticles for promoting neural differentiation of embryonic stem cells

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Statement of Purpose: The neural differentiation of embryonic stem cell (ESCs) has great practical applications for the treatment of neurodegenerative diseases. To overcome the disadvantages of natural glycosaminoglycans (GAG), GAG-mimicking polymers, that is designed glycopolymers containing functional units (such as saccharide and sulfonate groups) similar to natural GAG, were developed as potential chemical induction molecules for efficient neural differentiation.[1-3] Considering that the GAG related signal pathway for neural differentiation of ESCs originates in the cell membrane, we anticipate that the enrichment of GAG-mimicking polymers around cell membrane might further enhance the promotion effect. In this work, gold nanoparticle (GNP) were utilized as a vector to assemble polymers containing saccharide and sulfonate groups. The attractive interaction between GNP and cell membrane could further increase the utilization efficiency of functional units for neural differentiation, providing a new avenue for inducing neural differentiation of ESCs.

Methods: Monomers bearing saccharide and sulfonate groups, 2-methacrylamido glucopyranose (MAG) and sodium 4-vinylbenzenesulfonate (SS), were polymerized via the reversible addition-fragmentation chain transfer (RAFT) technique. The obtained polymers (pMAG and pSS) were reduced to get a thiol group terminal, which were further assembled into GNP with different pMAG/pSS feed ratios. The successful synthesis of thiol group terminated pMAG and pSS were confirmed by ^{1}HNMR and ultraviolet absorption spectra. DLS and visible absorption spectra indicated the polymers were successfully grafted onto the surface of GNP. The cell cytotoxicity test verified the nanocomposites had no significant effect on the growth and proliferation of mouse ESCs (mESCs). The neural differentiation of mESCs incubated with the GNP-pMAG/pSS nanocomposites were determined by immunofluorescence staining assay and quantitative polymerase chain reaction (qPCR) for neuronal marker β3-tubulin.

Results: The distributions of the neuronal marker β3-tubulin in cells at day 14 were analyzed by immunostaining for β3-tubulin and counterstaining with DAPI. As shown in Figure 1A, mESCs treated with GNP-pMAG/pSS nanocomposites or heparin showed stronger green fluorescence than the untreated cells (control group), demonstrating their promotion effect on neural differentiation. The degree of neural differentiation was further quantified by evaluating the gene transcriptional expression level of the differentiation marker β3-tubulin, which are expressed in the late stages of neural differentiation. As shown in Figure 1B, all nanocomposites samples have positive effect on neural differentiation, and the promotion effects were stronger than the natural heparin. Among all, GNP-M1S1 nanocomposite showed a highest expression level of β3-tubulin than other groups, indicating its excellent promotion effect on neural differentiation.

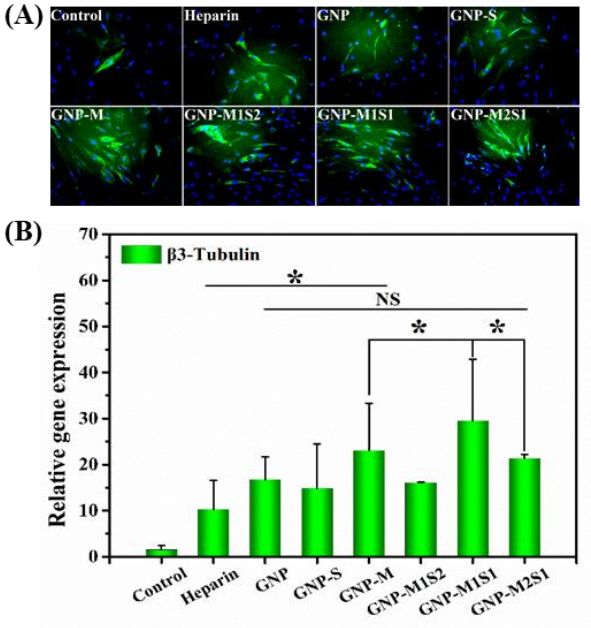

Figure 1. Neural differentiation of mESCs treated with heparin or GNP nanocomposites for 14 days. (A) Immunofluorescence images of mESCs treated with various molecules. GNP-S: GNP-pSS; GNP-M: GNP-pMAG; GNP-M1S2, GNP-M1S1 and GNP-M2S1: pMAG/pSS conjugated GNP with pMAG/pSS feed ratios of 1:2, 1:1 and 2:1 respectively. Green: β3-tubulin; Blue: DAPI. Scale bar: 100 μm. (B) β3-tubulin expression of mESCs in different groups. *$p < 0.05$.

Conclusions: In summary, nanocomposites constructed by combining GNP with polymers containing saccharide and sulfonate units were developed to mimic the function of heparin for neural differentiation. The prepared-nanocomposites showed obvious stronger promotion effect on neural differentiation than that of natural heparin, especially the nanocomposite GNP-M1S1. The nanocomposites proposed in this work provides possible opportunities for efficient neural differentiation of ESCs.

References:

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