Growth factor delivery by heparin complexed with a biocompatible polycation

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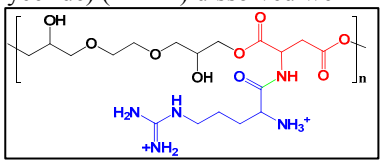
Statement of Purpose: Growth factor hold enormous therapeutic potential. However their applications are hampered by their short half life in the plasma, their large size, and potential side effect of systemic delivery. The objective of this project is to create biocompatible polycations that self-assemble with heparin polyvalently and control the release of heparin-binding growth factors. Polycations are very useful for many biomedical applications, but they are limited by their cytotoxicity. A unique strength of this research is the design of simple biocompatible polycations and the use of its self assembled conjugates with heparin to deliver many of the large family of heparin-binding growth factors.

Methods: The polymer was synthesized by direct condensation of a diglycidyl ester, aspartic acid and arginine. The resultant polymer, poly(ethylene argininylaspartate diglyceride) (PEAD) dissolved well in water. The biocompatibility of PEAD was assessed by 4 *in vitro* assays using baboon smooth muscle cells (SMCs): lactide dehydrogenase (LDH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Caspase-3, and live/dead. *In vivo* biocompatibility was evaluated by SC injection in rats followed by examination of the major organs and the implantation site. The charge of the polymer was measured by zeta potential. Self assembly of the delivery matrix was performed in PBS by simply mixing PEAD, heparin, and nerve growth factor (NGF). The binding between heparin and PEAD was measured by dimethylmethylene blue (DMB) assay. The loading efficiency of NGF was assessed by NGF enzyme-linked immunosorbent assay (ELISA) at various PEAD/heparin ratio. NGF release was monitored by ELISA as well. The bioactivity of the released NGF was investigated by measuring the neurite length of differentiated pheochromocytoma (PC12) cells with 3 controls: no NGF, bolus NGF, and heparin-protected NGF {[heparin:NGF]}. The amount of NGF in all NGF-containing groups were identical. The neurite length was quantified by measuring the 20 longest ones in 10 random views of each well (NIH Image J).



Results: PEAD (Mw = 26176, PDI = 2.28) had a zeta potential of 26 mV. In LDH and MTT assays, PEAD showed no toxicity at 2 mg/ml when using SMC medium as the control. At 5 mg/ml, there was approximately 20% difference with the control. Caspase-3 assay showed no toxicity up to 10 mg/ml. There was no difference between the dead-cell percentage between control and 10 mg/ml PEAD. The live-cell percentage displayed a 50% reduction between 10 mg/ml PEAD and control. *In vivo* data indicated that 1mg PEAD had no deleterious effects on the animal at 1, 3 or 28 days post-injection in 200g rats. This dosage was chosen because 1 mg PEAD can load at least 1 µg NGF. Coupled results from zeta potential and DMB assay indicated that the [PEAD :heparin] complex were neutral at mass ratio of 5/1 and the neutral complex precipitated out the highest amount of heparin at >99%. The 5/1 [PEAD:heparin] complex also loaded >99% of the NGF (500 µg PEAD, 100 µg heparin and 0.1 µg NGF. **Fig. 1**). The release of NGF had little burst and over a 3 week period, approximately 90% was released.

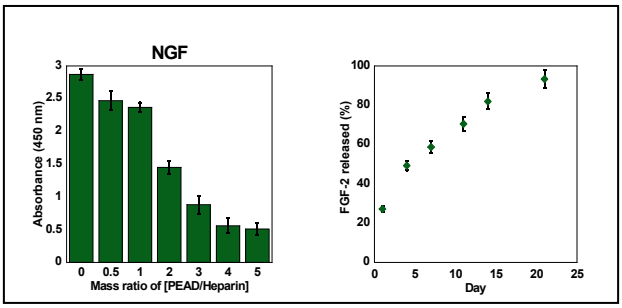
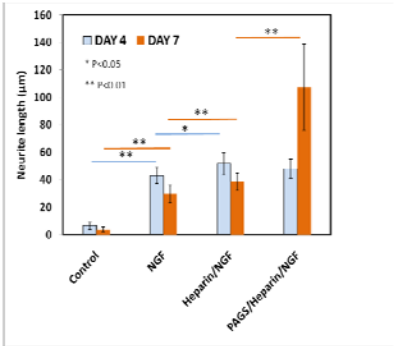


Figure 1. The loading and release profile of NGF in PBS at 37 °C.

When examined using differentiated PC12 cells, all NGF-containing groups displayed longer neurites than the basal medium. There were no difference between [heparin:NGF] and [PEAD:heparin:NGF] at day 4. However, at day 7, the controlled release group had significantly longer neurites ($P<0.01$). Furthermore, the controlled release group was the only one where the neurites kept extending during the 7 day culture period. Neurites in the two NGF-containing controls retracted after 4 days of culture indicating loss of NGF bioactivity.



Conclusions: We have designed and synthesized biocompatible polycations and used it to deliver NGF with heparin as the mediator. The Columbic interactions in the delivery matrix allowed NGF to be released up to 3 weeks. NGF bioactivity was preserved even better than soluble [heparin:NGF] complex. This platform can be used for many heparin binding growth factors. Other growth factor investigated includes FGF-2 and BDNF, which showed similar results.