Enhanced *in vivo* angiogenic activity of FGF-2 by a [polycation:heparin] complex

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Introduction

Growth factors regulate essential cell functions including survival, self renewal, differentiation and proliferation and hold great potential for regenerative medicine. However, their limited half life compromises the clinical utility significantly. A suitable delivery vehicle can greatly increase the efficiency and efficacy of growth factor therapy. Heparin, a highly sulfated polysaccharide, has strong affinity to various growth factors. In order to maintain its native property and function, we used a polycation to interact with heparin electrostatically without any covalent modification to heparin. [1] We examined the efficacy of FGF-2 delivery using this strategy *in vivo*.

Materials and Methods

A biocompatible polycation, poly(ethylene argininylaspartate diglyceride) (PEAD), self assembled with heparin and FGF-2 to form a coacervate [PEAD:heparin:FGF-2]. Prior *in vitro* studies demonstrated that [PEAD:heparin] complexcould encapsulate FGF-2 efficiently, control its release and maintain its bioactivity. [2] Here, saline, [PEAD:heparin], bolus FGF-2 (500 ng) or [PEAD:heparin:FGF-2] (500 ng FGF-2) was injected subcutaneously in the back of BALB/cJ mice. 1, 2 or 4 weeks post-injection, the animals were sacrificed. The tissues at the injection sites and the contralateral sites were harvested for hemoglobin quantification and immunohistological analysis to determine the extent of angiogenesis.

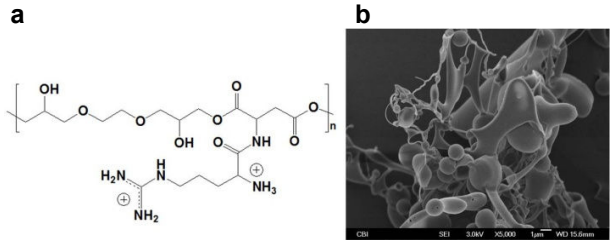

Fig. 1. (a) Chemical structure of PEAD (b) SEM micrograph reveals [PEAD:heparin] contains bead- and ribbon-like morphology. Bar scale 1 μm.

Results

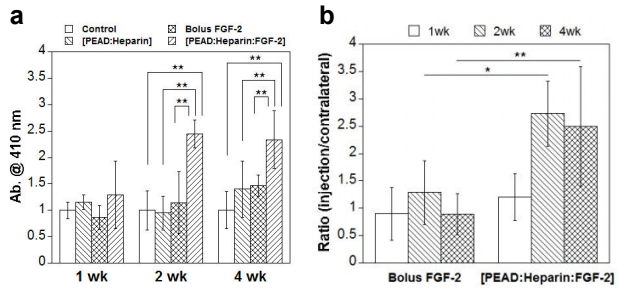

Fig. 2. (a) The quantification of hemoglobin at the injection sites 1, 2, and 4 weeks post-injection. The result suggests that [PEAD:heparin:FGF-2] group has significantly higher amount of hemoglobin compared to control and bolus groups. *p<0.05; **p<0.01 (b) The ratio of hemoglobin at the injection sites and the contralateral sites. The amount of hemoglobin at the injection sites is higher than that of the contralateral sites for the [PEAD:heparin:FGF-2] group, and the ratio is significant higher than that of bolus FGF-2. The result demonstrates that [PEAD:heparin] can deliver FGF-2 locally. [PEAD:heparin:FGF-2] alone induces no angiogenesis.

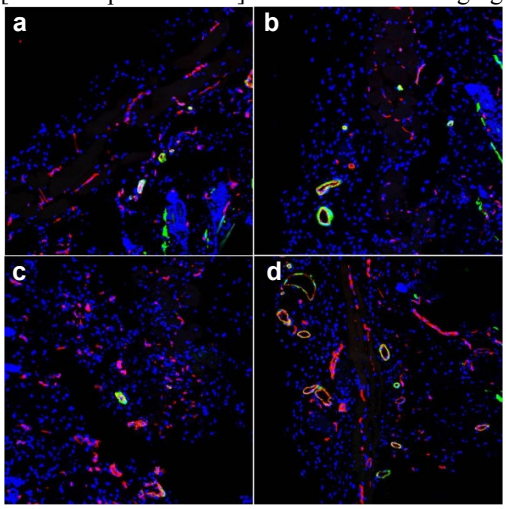

Fig. 3. Confocal imaging (20X) of the SC tissue: DAPI (blue) α-smooth muscle actin (green, mural cell) and CD31 (red, endothelial cell) (a) saline (control) (b) [PEAD:Heparin] (c) bolus FGF-2 and (d) [PEAD:Heparin:FGF-2]. The controlled delivery of FGF-2 induces significantly more blood vessel formation than all other groups; furthermore, many of the formed vessels are mature and lined by mural cells. Delivery vehicle alone induces no angiogenesis.

Discussion and Conclusions

Both qualitative and quantitative results demonstrate that [PEAD:heparin] coacervate is an excellent vehicle that greatly increases the angiogenic activity of FGF-2 over bolus injection. We are currently investigating the efficacy of this delivery platform in disease models in an effort to translate this into clinical reality.

References

[1] Zern BJ, Chu H, Wang Y. Control growth factor release using a self-assembled [polycation:heparin] complex. *PLoS One* 5(6): e11017.

[2] Chu H, Mason NS, Johnson NR, Wang Y. A [polycation:heparin] complex releases growth factors with enhanced bioactivity. *J Control Release* (under revision)

Disclosures

None