Controlled Release of rhVEGF from Heparin-Functionalized Nanoparticles

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Purpose: We present a new, facile method to prepare the heparin-functionalized nanoparticle system, which is composed of hydrophobic core (PLGA), hydrophilic surface layer (Pluronic F-127), and heparin, the functional moiety, that is entrapped and stabilized in the surface hydrogel layer. This system is noble since 1) all three components are proven-biocompatible materials by FDA, and 2) more importantly, no chemical modification of any component, including heparin, is employed.

Methods: The heparin-functionalized PLGA nanoparticles (HEP-PLGA NPs) were prepared by a spontaneous emulsion solvent diffusion method, and their size, polydispersity, and surface charge were analyzed. ^{1}H NMR and anti-factor Xa assay on HEP-PLGA NPs were carried out to calculate constitutional ratio in nanoparticles. The in vitro release rate of lysozyme as well as rhVEGF from HEP-PLGA NPs was analyzed. To evaluate the angiogenic potential of HEP-PLGA NP system, the fibrin gel containing HEP-PLGA NPs, in which VEGF was loaded, was implanted into the subcutaneous pockets of mouse, and assayed in 2 weeks.

Results / Discussion: HEP-PLGA NPs were prepared by slowly adding the organic phase into the aqueous solution containing heparin. By increasing the amount of heparin in aqueous phase during the preparation of nanoparticles, the zeta potential value decreased significantly, whereas the size and the polydispersity of nanoparticles were varied little (Figure 1). The amount of heparin incorporated was controllable up to 4.7 wt %. The release of lysozyme from the HEP-PLGA NPs showed a linear and complete release profile, maintaining their bioactivity. The release rate was also adjustable by the heparin amount in the nanoparticle. The release of VEGF from the nanoparticles showed a linear, and more prolonged profile (~3%/day) without initial burst (Figure 2). Immunohistological assay in 2 weeks after implantation showed that the fibrin gel with VEGF-loaded nanoparticle noticeably increased capillary density around the implant compared to the fibrin gel with the same amount of VEGF loaded as a solution.

Conclusions: The HEP-PLGA NPs, having the heparin molecules on the surface, were prepared without any chemical reaction. The release of VEGF from the nanoparticles showed a linear profile without initial burst over one month. The fibrin gel-nanoparticle composite for the local delivery of VEGF showed therapeutic potential for angiogenesis in a mouse implantation model. Thus, this HEP-PLGA NPs can be employed a growth factor delivery component in tissue engineering scaffolds as well as a sustained release system of various heparin-binding growth factors.

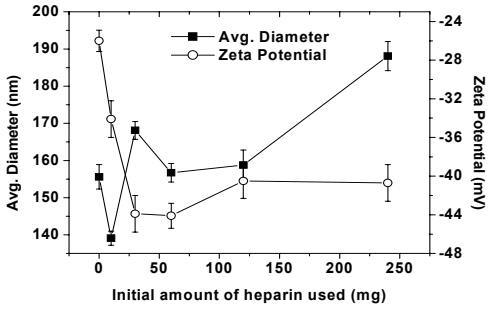

Figure 1. The size and the surface charge of the heparin-functionalized PLGA nanoparticle by varying the amount of heparin in aqueous phase during preparation.

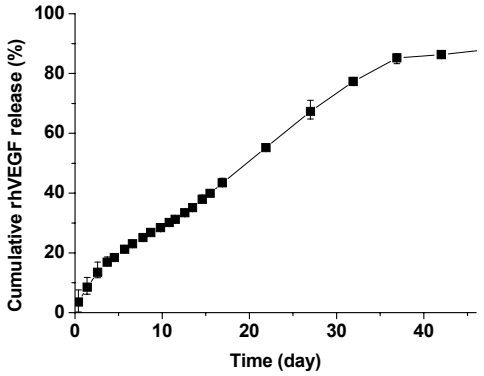

Figure 2. Cumulative VEGF release from the heparin-functionalized PLGA nanoparticle.

References

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