A New Injectable Hydrogel Induces Angiogenesis through Controlled Release of Basic Fibroblast Growth Factor

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Statement of Purpose: In the present study, we hypothesized that self-assembly hydrogels comprising of peptide-amphiphile (PA) and basic fibroblast growth factor (bFGF) can be used to fabricate tissue engineering scaffolds to induce angiogenesis and used it for feasibility of prevascularization by the bFGF release from scaffold in improving efficiency of tissue regeneration.

Methods: PA was synthesized by standard solid phase chemistry that ends with the alkylation of the NH_2 terminus of the peptide [1]. The chemical structure of PA contain RGD (arginine-glycine-aspartic acid), a Glu (Glutamic acid) residue, four Alanine (Ala) and three Glycine (Gly) residues (A_4G_3), followed by an alkyl tail of 16 carbons. A 3D hydrogel was formed by mixing bFGF suspensions with dilute aqueous solutions of PA. Scanning electron microscopy (SEM) observation revealed the formation of fibrous assemblies with an extremely high aspect ratio and high surface areas with mean diameter of 20 nm. *In vitro* and *in vivo* release profile of bFGF from 3D hydrogel was investigated while angiogenesis induced by the released bFGF was assessed. For the evaluation of angiogenesis induced with these injectable scaffolds, 50 μl of four doses of bFGF solutions (0.04, 0.2, 0.6, and 1 μg/μl) and 50 μl of PA solutions were subcutaneously injected at the same time into the back of rats. At 1, 3, 7, 10, 14, 21, and 28 days post-treatment, the rats were sacrificed by an overdose injection of anesthetic and the skin including the injected site (2 × 2 cm^2) was carefully taken off for the subsequent biological examinations. The angiogenesis of bFGF was estimated by determining the amount of tissue hemoglobin as a marker of angiogenesis.

Results/Discussion: A 3d hydrogel was formed after injection of bFGF with PA into the back of rats. When bFGF was injected together with PA solution, capillaries were newly formed at the injected site. bFGF injection alone did not contribute to vascularization, and the tissue appearance was similar to that of PA injection alone. Figure 1 shows the time course of angiogenesis induced by free bFGF, PA solution, and bFGF injection with PA. The injection of bFGF solution did not increase the amount of hemoglobin at the injection site over the time range studied and the amount of tissue hemoglobin was similar to that of PA solution alone or untreated, normal mice. However, the injection of bFGF together with PA solution induced significant angiogenesis. The amount of tissue hemoglobin notably increased within 1 day of injection and the significantly increased level was retained over 28 days, and thereafter returned to the initial level of tissue hemoglobin.

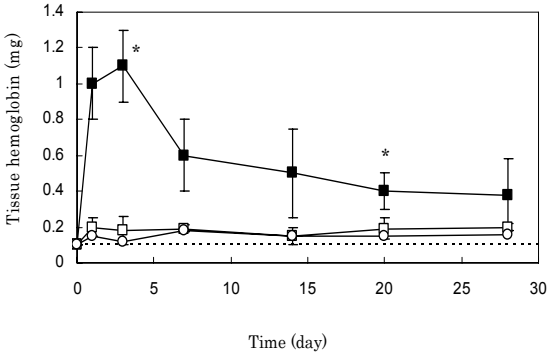

Figure 1. Time course of neovascularization induced by subcutaneous injection of bFGF alone (□), PA (○), and bFGF with PA (■). The concentration of bFGF is 0.2 μg/μl. The dotted line indicates the amount of tissue hemoglobin in the corresponding area of untreated, normal rat. * $p < 0.05$, significant against the value of group injected with PA.

Conclusions: These results strongly suggest that the angiogenesis in advance induced by the controlled release of bFGF from bFGF-incorporated PA played an important role in creating an environment suitable for the survival and activity of transplanted cells for further applications in tissue regeneration.

References:

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