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Enhanced angiogenesis by multiple release of platelet-rich plasma contents and basic fibroblast growth factor from gelatin hydrogels

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ABSTRACT

The objective of this study is to evaluate the angiogenic effects induced by biodegradable gelatin hydrogel granules incorporating mixed platelet-rich plasma (PRP) growth factor mixture (PGFM) and bioactive basic fibroblast growth factor (bFGF). The PRP was prepared by a double-spinning technique for isolating animal bloods, followed by treatment with different concentrations of calcium chloride ($CaCl_2$) solution. The $CaCl_2$ solution treatment activated the platelets of PRP, allowing the release of various growth factors, such as platelet-derived growth factor (PDGF)-BB, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-β_1, and epithelial growth factor (EGF). In the PRP treated with different $CaCl_2$ solutions, high amounts of representative platelet growth factor, PDGF-BB, VEGF, EGF, and TGF-β_1 were detected in the $CaCl_2$ concentrations of 1, 2, and 4 wt.% compared with higher or lower ones. The PRP treated was impregnated into gelatin hydrogel granules freeze-dried at 37 °C for 1 h, and then the percentage of PGFM desorbed from the gelatin hydrogel granules was evaluated. The percentages of PDGF-BB, VEGF, EGF, and TGF-β_1 desorbed tended to decrease with decreasing $CaCl_2$ concentration. Taken together, the $CaCl_2$ concentration to activate PRP for PGFM release was fixed at 2 wt.%. In vitro release tests demonstrated that the PGFM was released from the gelatin hydrogel granules with time. For the gelatin hydrogels incorporating PGFM and bFGF, the time profile of PDGF-BB or bFGF release was in good correspondence with that of gelatin hydrogel degradation. The gelatin hydrogel granules incorporating mixed PGFM and bFGF were prepared and intramuscularly injected to a mouse leg ischemia model to evaluate the angiogenic effects in terms of histological and laser Doppler perfusion imaging examinations. As controls, hydrogel granules incorporating bFGF, PGFM, and platelet-poor plasma were used for the angiogenic evaluation. The number of blood vessels newly formed and the percentage of anti-α-smooth muscle actin antibody-positive cells increased around ischemic sites injected with the gelatin hydrogel granules incorporating mixed PGFM and bFGF, in marked contrast to other control groups. The blood reperfusion level of ischemic tissues was enhanced by the hydrogel granules incorporating mixed PGFM and bFGF, whereas no enhancement was observed for other groups. It is concluded that the dual-release system of PGFM and bFGF from gelatin hydrogel granules shows promise as a method to enhance angiogenic effects.

1. Introduction

Several treatments for therapeutic angiogenesis have been reported to demonstrate experimental and clinical potential. A number of clinical trials of gene therapy and bone marrow cells transplantation have been carried out. However, the biosafety of genetic materials is not always certain [1–3]. In addition, the invasiveness required to harvest bone marrow cells and the lack of scientific knowledge and related technology for bone marrow cells may be potential risks for cell therapy [4,5]. Another option is to make use of growth factor proteins, which enable cells to naturally induce their biological potential for natural angiogenesis. If the growth factor can be used in vivo with the biological activity remaining, growth-factor-induced angiogenesis is a realistic prospect.

A drug delivery system (DDS) is one plausible technology for enhancing the in vivo biological activity of growth factors. Biodegradable gelatin hydrogels have been designed and prepared for the controlled release of bioactive basic fibroblast growth factor (bFGF) [6], transforming growth factor (TGF)-β_1 [7], platelet-derived growth factor (PDGF)-BB [8], bone morphogenetic protein (BMP)-2 [9–11], and hepatocyte growth factor (HGF) [12,13]. For example, bFGF can be released from gelatin hydrogels, demonstrating the therapeutic potential of angiogenesis in animal models of ischemic legs and hearts or the angiogenesis-induced enhancement of therapeutic efficacy of cell transplantation [14–21]. In addition, a clinical trial has been started to confirm the safety

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