Enhanced Vascularization by Controlled Release of Platelet-Rich Plasma Impregnated in Biodegradable Gelatin Hydrogel

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Background. **Platelet-rich plasma (PRP) contains numerous growth factors that have angiogenic activities. However, the PRP-induced angiogenesis is limited by the short half-life period of growth factors. A new drug delivery system of biodegradable gelatin hydrogel was designed to achieve the controlled release of growth factors in PRP. The purpose of this study is to demonstrate the therapeutic efficacy of slow-release of PRP in the inducing of angiogenesis for critical ischemia.**

Methods. **The PRP was prepared from the whole blood of inbred rats. Thirty-two rats underwent excision of the left femoral artery and its branches to create critical limb ischemia. The rats were randomized into four groups (n = 8 each): no treatment (control), intramuscular injection of platelet-poor plasma (PPP), PRP only, or a combination of PRP and gelatin hydrogel (PRP+Gel). Four weeks after the treatment, angiogenesis was evaluated by laser doppler, microangiogram, and immunohistology.**

Results. **The resultant number of platelets for PRP was higher than that of PPP ($p < 0.01$). The concentrations of vascular endothelial growth factor, transforming growth factor-β1, and platelet-derived growth factor-BB were significantly higher in PRP animals than in PPP ($p < 0.01$). Although the PRP group improved tissue blood flow (82.7% ± 6.2%) compared with the control group or PPP group (69.6 ± 12.2 or 72.2 ± 11.8%, $p < 0.05$), the improvement of blood flow in the PRP+Gel group was significantly better (95.1% ± 8.0%, $p < 0.05$) than in the PRP group. Angiographic score in the PRP+Gel group was significantly higher than that in the control, PPP, and PRP groups (8.6 ± 2.1 versus 3.8 ± 0.8, 3.7 ± 0.6, and 5.6 ± 1.5, respectively; $p < 0.01$). Capillary density also increased immunohistologically in the PRP+Gel group when compared with the control, PPP, and PRP groups ($p < 0.01$).**

Conclusions. **A controlled release system of PRP was effective in inducing angiogenesis for critical ischemia. The biodegradable gelatin hydrogel incorporating PRP as applicable could possibly be used to treat for patients with ischemic cardiomyopathy.**

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In recent years, angiogenesis research has made remarkable progress in the fields of ischemic heart and limb diseases. Various therapeutic angiogenesis techniques including gene therapy using potent growth factors have been reported, some with positive results in animal studies or clinical trials [1-3]. However, most of these approaches were based on the biological effect of a single recombinant angiogenic molecule. For the critical ischemic cases, it seems to have be limited because the establishment of stable and functional blood vessel networks is a complex process and requires several angiogenic factors to stimulate the vessels sprouting and remodeling the primitive vascular network [4]. Moreover, there are some major concerns about adverse effects of high-dose single recombinant growth factors, as well as immune and inflammatory reactions shown in previous studies [5].

Conversely, it had been described previously that a combination of growth factors synergistically induced angiogenesis and long-lasting functional vessels compared with single growth factor [6, 7]. Platelet-rich plasma (PRP) is an autologous concentration of platelets in a small volume of plasma and contains biologically determined ratios of various autologous growth factors in high concentrations [8]. It is also cost saving, biologically safe, and has conventional availability. Moreover, it has been already used clinically in the dentistry, maxillofacial surgery, and plastic surgery fields for the application of regenerating damaged tissue [8, 9].

However, application of PRP is currently limited because of its short half-life period [8, 10]. Since the PRP

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Abbreviations and Acronyms

IEP	= isoelectric point
LDPI	= laser doppler perfusion imaging
PBS	= phosphate-buffered saline
PDGF-BB	= platelet-derived growth factor-BB
PPP	= platelet-poor plasma
PRP	= platelet-rich plasma
TGF-β1	= transforming growth factor-β1
VEGF	= vascular endothelial growth factor
vWF	= von Willebrand factor

and growth factors dissolve into blood stream in just a couple of days after injection, it is difficult to maintain its presence in the tissue long enough for the promotion of effective vascularization [8]. To resolve this disadvantage, we employed a novel drug delivery system utilizing biodegradable gelatin hydrogel [11]. The biodegradable gelatin hydrogel can control the timing of the release of the growth factors to ischemic regions. Because platelets are activated by impregnation of the PRP into the biodegradable gelatin hydrogel, growth factors are secreted from the platelets [12]. The secreted growth factors are immobilized in the hydrogel through electrical and physicochemical interaction with gelatin molecules (Fig 1). Degradation of the hydrogel impregnated with growth factors allows for slow release of growth factors into the tissue over a period of about 2 weeks after the injection of PRP and biodegradable gelatin hydrogel into the ischemic tissue, resulting in effective angiogenesis [12, 13].

The purpose of this study was to verify the effectiveness of the controlled release of PRP using the biodegradable gelatin hydrogel in inducing angiogenesis for critical limb ischemia.

Material and Methods

Experimental Animals

All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals," prepared by the National Academy of Science and published by the National Institute of Health (NIH Publication 86-23, revised 1985). In addition, this study protocol was approved by the Animal Ethics Committee of Nippon Medical School (approval number 21-145), and was performed according to the "Guidelines for Regulation of Animal Experiments" of Nippon Medical School. Male Wistar rats (Sankyo Labo Service, Tokyo, Japan), weighing 380 to 400 g and aged 10 to 12 weeks were used for the experiments. Animals were maintained in a room controlled for temperature and light and were provided food and water ad libitum. These isogenic rats were used as donors and recipients of PRP to simulate autologous implantation.

Preparation of PRP and Platelet-Poor Plasma

Whole blood (7 to 10 mL) was preoperatively drawn from an inbred male Wistar rat through cardiac puncture with an 18G needle (Terumo, Tokyo, Japan) into tubes containing 3.8% sodium citrate, under general anesthesia

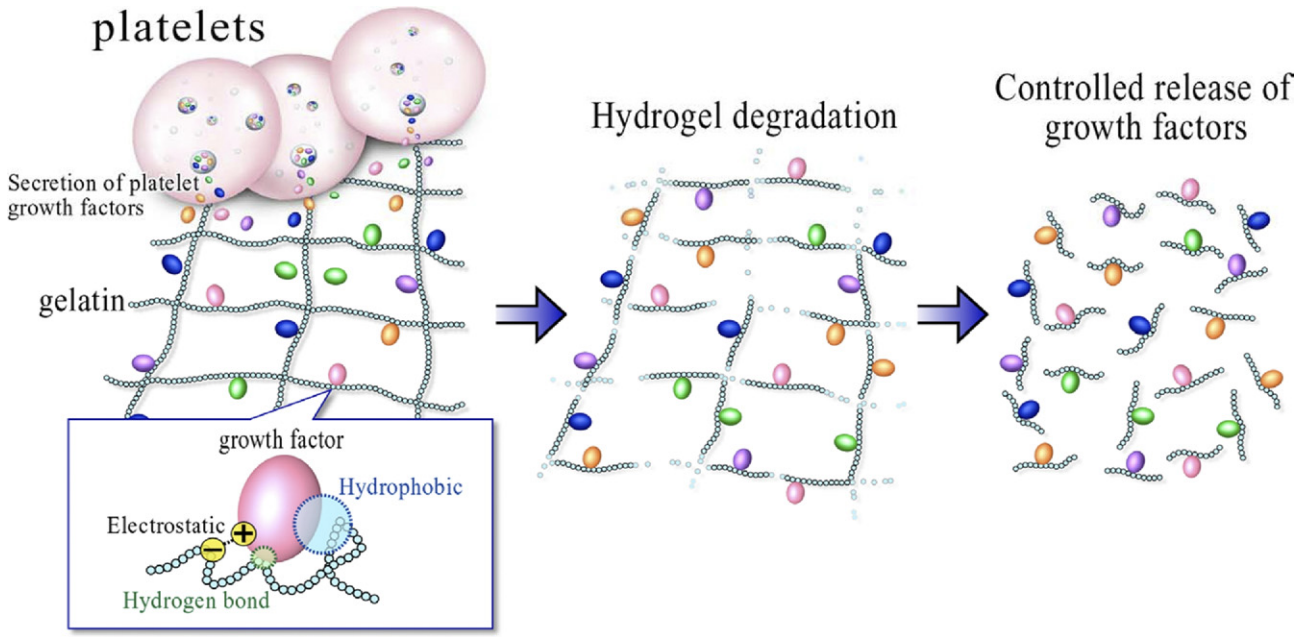

Fig 1. Schematic illustration of the controlled release of growth factors contained in platelet-rich plasma (PRP). When platelets are activated by contact with gelatin hydrogel molecules, growth factors are released from the platelets. The growth factors are immobilized in the gelatin hydrogel through electrical and physiochemical interaction with gelatin molecules. The immobilized growth factors are released as a result of hydrogel degradation.