Application of Platelet-rich fibrin for Acute Myocardial Infarction Treatment

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Abstract

Acute myocardial infarct is a major high mortality cause in heart attacks. The aim of this study was to evaluate the repair of acute myocardial infarct damage following the implantation of platelet-rich fibrin (PRF) in a one-step procedure. We demonstrated the improvement of acute myocardial infarct damages with different concentrations of platelet-rich fibrin extract by direct-myocardial injections. Platelet-rich fibrin contains several growth factors mainly include platelet derivative growth factor (PDGF–A,B), transforming growth factor(TGF-β), vascular endothelial growth factor (VEGF) and epidermal growth factor(EGF). Platelet growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, cell regeneration and cell proliferation. Platelet-rich fibrin extracts implantation for acute myocardial infarct show optimally in infarct area decrease and new angiogenesis. Fibrosis formation was unclear in myocardium infarct area. Heart function measurement demonstrated improvement of left ventricular ejection fraction (LVEF) and fraction shortening (FS) in platelet-rich fibrin extract groups.

Key Words: Acute myocardial infarct、 biomaterial 、 Growth factor 、heart attacks 、 Platelet-rich fibrin

1. Introduction

Acute myocardial infarction is a state of inadequate blood perfusion induced by coronary artery blockage. The incidence of cardiogenic shock complicating acute myocardial infarction (AMI) ranges from 5% to 10% 〔1〕. Myocardial infarction (MI) focuses on the myocardium damages. Within 20 to 40 minutes of an occlusion irreversible myocardial cells damage occurs. The main changes are necrosis or apoptosis of myocardial cells. Damages of acute myocardial infarction result in left ventricular dilatation and remodeling、congestive heart failure and poor clinical outcomes 〔2〕. The prognosis of therapy for acute myocardial infarction has improved over time because of aggressive reperfusion strategies, the mortality rate from cardiogenic shock still remains very high 〔3〕.

The development of bioactive surgical additives tried to regulate inflammation and increasing healing is difficult and complicate. In each intervention, the tissue remodeling and the consequences on healing and tissue repair is a challenge 〔4,5〕. Platelet-rich fibrin is a new generation platelet derivative product. Fibrinogen is the

final substrate of all coagulation reactions. Fibrinogen is a soluble protein and is transformed into an insoluble fibrin by thrombin while the polymerized fibrin gel constitutes the basic matrix of the injured site 〔6,7,8〕. Platelet-rich fibrin is a specifically developed biomaterial for use in oral, maxillofacial surgery and tissue healing 〔9,10,11〕. Fibrin adhesives are already used in cardiothoracic and vascular surgery. These adhesives are successfully used for the sealing of diffuse microvascular bleeding through spray application 〔4〕. Platelet-rich fibrin is collected from no-anticoagulant blood by centrifugation and the preparation of PRF is simple and can be completed during one procedure 〔12〕. Platelet-rich fibrin contains several growth factors mainly include platelet derivative growth factor (PDGF–A,B), transforming growth factor(TGF-β), vascular endothelial growth factor (VEGF) and epidermal growth factor(EGF)〔13,14,15〕. Platelet growth factors have exhibited chemotactic and mitogenic properties 〔16〕that promote and modulate cellular functions involved in tissue healing, cell regeneration and cell proliferation 〔16,17,18〕. PRF contains not only a number of GFs, but also cytokines and inflammatory mediators 〔19〕. A study has reported that GFs may promote re-epithelialization during skin wound healing〔20〕. The aim of this study was to evaluate the protection of myocardium defects of acute myocardial infarct following the implantation of PRF extract in a one-step procedure in pig.

2.1 AMI induction

Experimental animals were anesthetized by intramuscular injections with atropine、Xylazine (Bayer) and Zoletil-50 (Virbac) and maintained with an inhalation of 2 % isoflurane during the procedures. They were intubated endotracheal tubes with positive pressure ventilation with room air and oxygen using a ventilator (ADS-1000). Electrocardiogram (ECG, NIHON KOHDEN CORPORATION) monitor was connected to the four limbs. The heart was exposed through left-thoracotomy and the mid-LAD was doubly ligated with 2-0 PDS suture just after the first diagonal branch for 30 minutes. Myocardial infarct-ischemia area is confirmed by observing a rapid whitish discoloration of the anterior wall of left ventricle and myocardial infarction was also confirmed by S-T segment elevation of ECG following the procedures (Figure 1). The muscle and skin were then closed in layers. The animals were sacrificed on day 7 following AMI induction to estimate the efficiency of PRF protection.

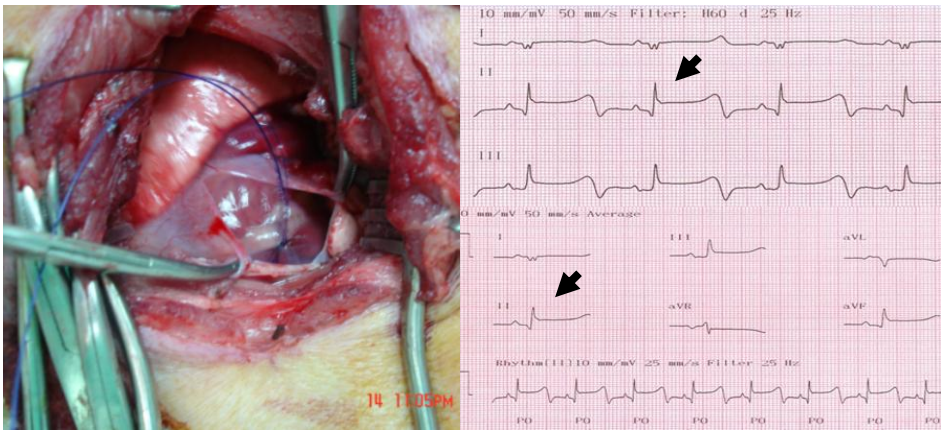

Figure 1: Induction of acute myocardial infract in pig. Descending branch of left coronary artery (LAD) ligation was finished with 2-0 PDS suture by left thoracotomy (A). The whitish appearance was noticed and the S-T segment elevation (arrow head) appeared after LAD branch ligation in EKG (B).

2.2 Platelet-rich fibrin preparation and delivery

The PRF was prepared using the technique described by Dohan et al 〔4〕. Immediately after 20 cc of blood had been drawn from the jugular vein of each animal before surgery, the blood sample was centrifuged〔21〕. The PRF gel was formed in the middle layer of the tube where platelets concentrations are largely located 〔22〕. Gel was removed for PRF content extraction immediately. The PRF extract is prepared for implantation to myocardium of AMI. Surgery was performed under general anesthesia by a senior surgeon (S-C Lin). PRF extract was implanted into damaged myocardium directly (Figure 2). PRF extract is thought to release growth factors locally for several days, inducing accelerated tissue repair 〔23〕.

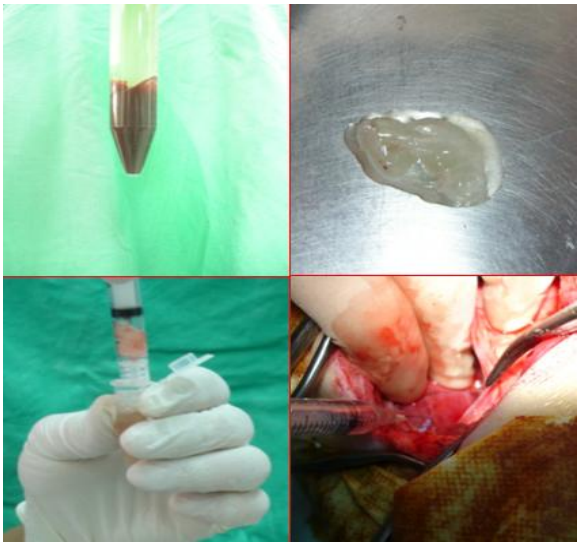

Figure 2: Procedures of PRF isolation and implantation: The PRF extract was isolated from blood by centrifugation. The gel of PRF was formed and PRF extract was

collected with 5mL syringe compression. The PRF extract was injected into peri-myocardial infarct area with 5 injections.

2.3 Functional assessment by echocardiography

Transthoracic echocardiography was performed preoperatively and on day 7 after AMI induction under anesthesia as described previously, using a commercially available echocardiographic system (UF-750XT) equipped with two 3.5-MHz and 6.5-MHZ transducers for animals (FUKUDA Denshi Co. Hongo, Bunkyo-Ku, Tokyo, Japan). With the animals in a right recumbency position, left ventricular internal dimensions. End-systolic diameter (ESD) and end-diastolic diameter (EDD)] were measured. The LV ejection fraction (LVEF) and LV fraction shortening (LVFS) were measured (Figure 3). All measurements were performed by an animal cardiologist blind to treatment and non-treatment groups.

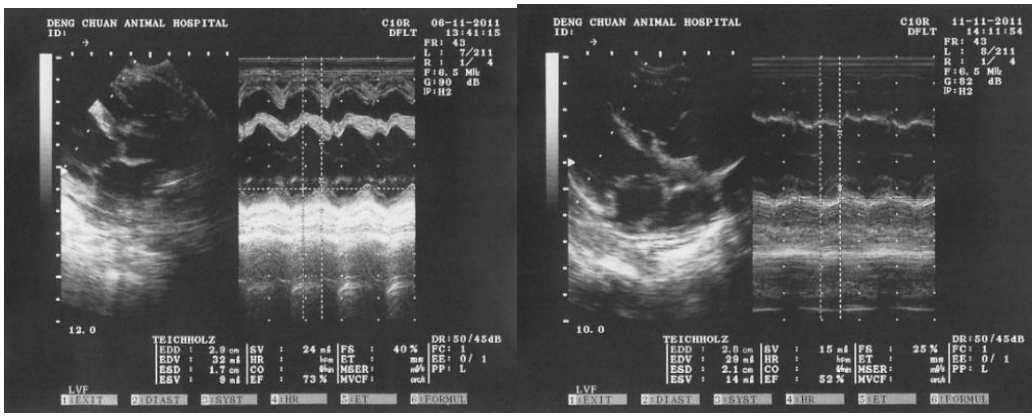

Figure 3: Effects of PRF implantation on the left ventricle function of acute myocardial infarct (AMI) in pig. Estimation of LV functions before of AMI induction (A) and PRF implant therapy in AMI (B) was determined by echocardiography.

2.4 New vessel density in infarct area

The gross heart was sectioned to 6 pieces with one centimeter interval after animal sacrifice. Pathological sections were obtained from the infarct, junctional, and non-infarction areas (Figure 4). The sections were processed with hematoxylin & eosin (HE) stain. Three sections of the infarct areas were chosen for each pig and three randomly selected high-power fields (HPF) (×200) were analyzed. The arterioles (≤100 μm in diameter) were chose and the mean number per HPF for each animal was then determined by summation.

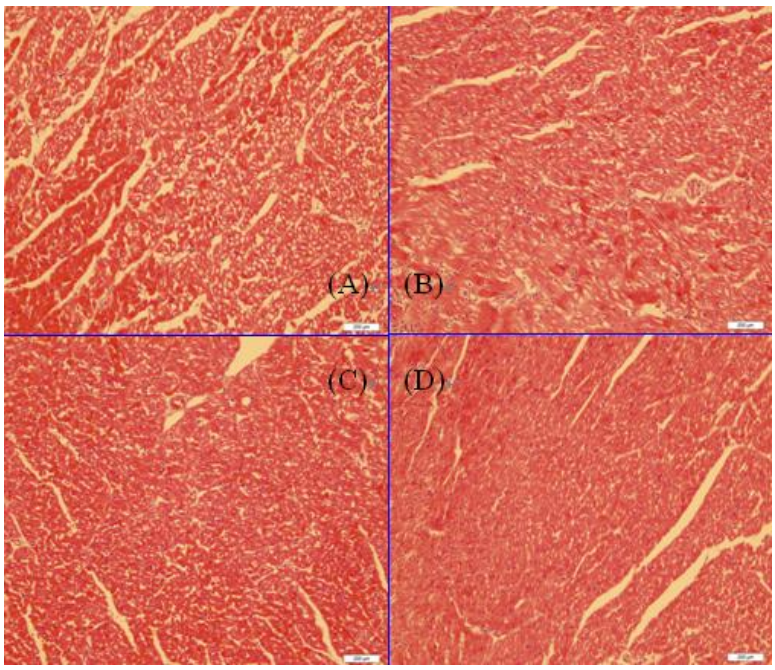

Figure 4: Pathologic examination of myocardial damages by artificial AMI with different treatment: The PRF extract implant groups remain better myocardium structure than AMI alone group. A lot of vacuoles were noticed in myocardiocyte of AMI alone group. (A- AMI alone, B- AMI plus 0.025cc/kg PRF, C- AMI plus 0.05cc/kg PRF, D- AMI plus 0.1cc/kg PRF)

2.5 Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA). A probability of less than 0.05 was considered to be statistically significant.

3. Result

Platelet-rich fibrin extracts implantation for acute myocardial infarct show optimally in infarct area decrease and new angiogenesis. Fibrosis formation was unclear in myocardium infarct area and left ventricular function measurements demonstrated the improvement of left ventricular ejection fraction (LVEF) and fraction shortening (FS) in platelet-rich fibrin extract for AMI groups.

3.1 Functional assessment results by echocardiography:

The experiment animals were divided into four groups as described. The control group show serious myocardial damages in clinic measurement. EF and FS values were decreased obviously. Both 0.025cc/kg and 0.05cc/kg PRF extract groups show better left ventricular function performance than AMI alone group and 0.1cc/kg PRF extract group. The 0.025cc/kg group demonstrated the best function improvement in all groups.

The 0.025cc/kg group and 0.05cc/kg groups were completely recovery from AMI damages. The EF and FS values are better than before AMI induction (Figure 5). PRF provides the growth factors and accelerates the recovery of myocardium; unsuitable concentration is harmful to damage area. The 0.1cc/kg PRF extract group demonstrated the different result to another two PRF extract groups.

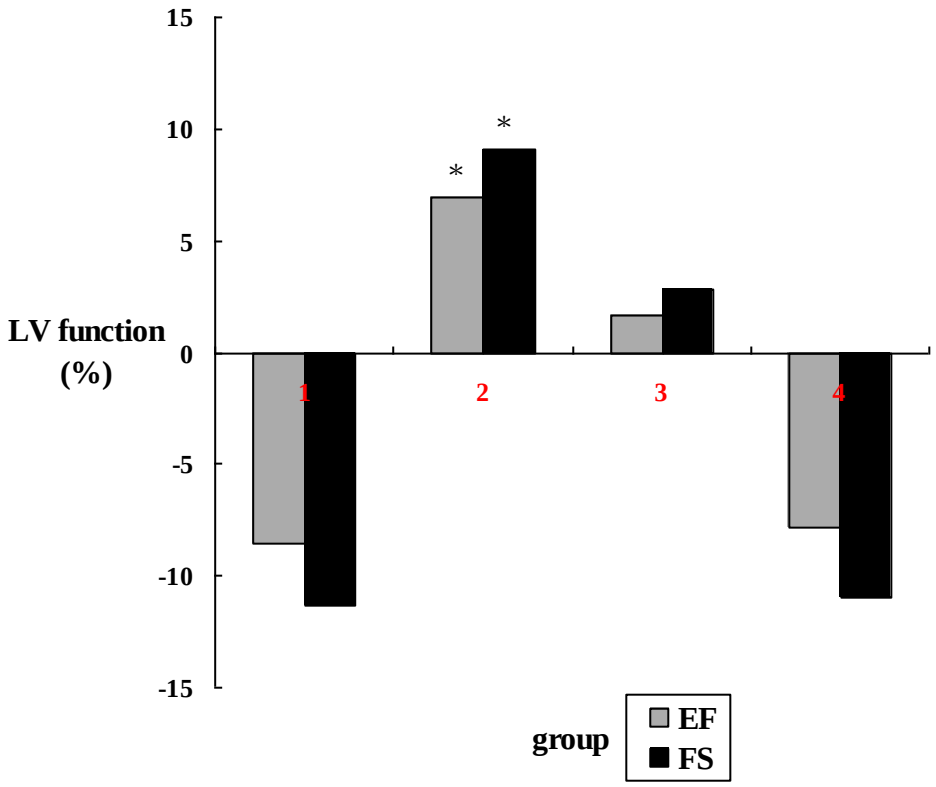

Figure 5: Ejection fraction (EF) and Fraction shortening (EF) assessment results by echocardiography: Both 0.025cc/kg and 0.05cc/kg PRF extract groups show better left ventricular function performance than AMI alone group and 0.1cc/kg PRF extract group. The higher concentration (0.1cc/kg) of PRF extract did not demonstrate better LV function. (1)- AMI alone, (2)- AMI plus 0.025cc/kg PRF, (3)- AMI plus 0.05cc/kg PRF, (4)- AMI plus 0.1cc/kg PRF). ＊ $P < 0.05$.

3.2 Angiogenesis in infarct area and myocardial recovery with PRF

The arterioles (≤100 μm in diameter) were chose and the mean number per HPF for each animal was then determined by summation. The angiogenesis has a trend to higher concentration of PRF. The 0.1cc/kg group demonstrates the best new vessels formation. The AMI alone group has the least new vessels formation (Figure 6). The damages of AMI caused myocardiocytes apoptosis and necrosis, resulting in cell vacuoles and fibrosis formation. The PRF contains several growth factors could prevent

cells death via increasing cell growth to replace and angiogenesis to provide enough oxygen.

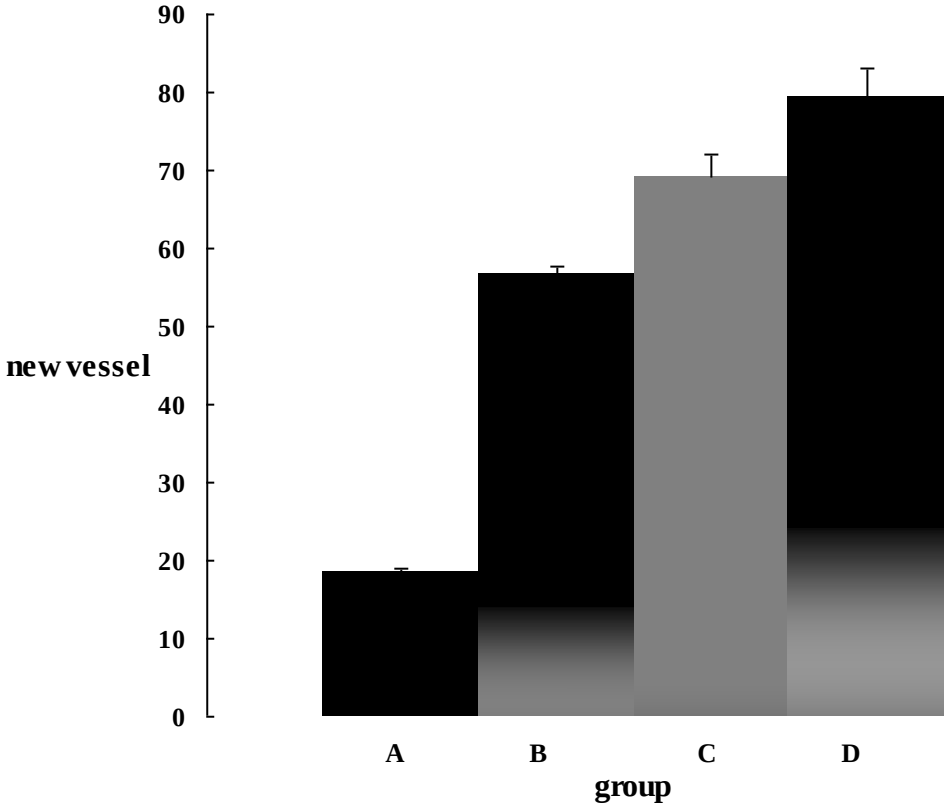

Figure 6: Angiogenesis in infarct area: The arterioles (≤100 μm in diameter) were chosen The PRF extract implant groups show better new vessel formation than AMI alone group. There is a trend that the higher concentration PRF demonstrates better angiogenesis. (A- AMI alone, B- AMI plus 0.025cc/kg PRF, C- AMI plus 0.05cc/kg PRF, D- AMI plus 0.1cc/kg PRF)).

4. Discussion

The incidence of cardiogenic shock complicating acute myocardial infarction ranges from 5% to 10%. The high mortality remains even with the progress of angioplasty (PTCA or sten implantation). The affected myocardium was induced cells apoptosis and necrosis could be the major reason. Platelet rich fibrin is a simple and immunologically safe source of growth factors. The middle layer contains a concentrate of the patient's platelets after blood centrifugation 〔24〕. These platelets have granules that contain growth factors that affect every cell and the formation of every tissue involved in the wound healing and regeneration of soft tissue and bone 〔25〕. In our results, platelet rich fibrin extracts demonstrated optimal improvement in acute

myocardial infarct damages model, including infarct area decrease, less fibrosis formation, more angiogenesis and better left ventricular function performance. Several growth factors were secreted via platelet activation 〔11〕. These growth factors protect myocardium from apoptosis and necrosis by ischemia and reperfusion damages. These elements mainly include platelet-derivative growth factor (PDGF-A,B), vascular endothelium growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-β1), epidermal growth factor (PD-EFG), and insulin-like growth factor1,2 (IGF-1,2). Vascular endothelium growth factor (VEGF) could accelerate the functions of cells growth, differentiation, new blood vessel growth and anti-apoptosis. Epidermal growth factor (PD-EFG) has the potential of cell growth and cytokine secretion, increases cell differentiation. Platelet-derivative growth factor (PDGF-A, B) demonstrates the functions of potent cell growth, recruitment, blood vessel growth, granulation and matrix formation (collagen) 〔26〕. Growth factors diffuse quickly from the PLT gel and may be available to the surrounding tissues. PDGF-AB, TGF-β 1, EGF, and VEGF content increased gradually over time when the PLT gel was left with the releasate 〔13〕. PRF extracts could secret different concentration of growth factors in different interval. Our results show the increase of new angiogenesis and less fibrosis formation in 5 minutes extraction. Content in the extract reached mean values of 29.29, 43.06, 0.38, and 0.18 ng at 5 minutes for PDGF-AB, TGF- 1, VEGF and EGF 〔13〕. The cardiomyocyte protection may be attributed, at least in part, to enhanced neovascularization in the wound beds, which is likely due to sustained delivery of GFs contained in PRF extract. PDGF-A, B provide new ECM for cell ingrowth and supply oxygen and nutrients for cell metabolism 〔27〕. The enhanced neovascularization by sustained delivery of GFs contained in PRP has been also reported in a previous study demonstrating that sustained release of GFs in ischemic hind limbs in mice 〔28〕. Neovascularization process involves MMP 〔12〕. MMP-2 and MMP-9 mediate neovascularization by endothelial cell migration as they degrade ECM 〔29,30,31〕. MMPs are localized on endothelial cells. MMP expression is stimulated by GFs such as VEGF and FGF 〔32,33〕. Our study indicates that the acute myocardial infarct damages could be relieved with PRF. The application of PRF represents a one-step myocardium protection and repair with potentially favorable results.

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