

Rationale of Platelet Gel to Augment Adaptive Remodeling of the Injured Heart

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Abstract: Cardiac pathologic events including; myocardial infarction, viral infection and hypertrophy, and aging, may trigger maladaptive remodeling of the myocardium. Maladaptive remodeling results in diastolic and systolic dysfunction, myocyte loss, and malformation of the extracellular matrix. It is proposed that platelet gel applied to the site of myocardial injury may provide the proper cytokines, growth factors, and chemokines to promote adaptive remodeling. The hypothesis is that platelet gel concentrates may provide a temporary and local hyperphysiologic concentration of platelet secretory factors that may initiate adaptive myocardial healing. Autologous platelet gel can be

derived from concentrated platelets activated and induced to secrete cytokines, growth factors, and chemokines with an array of stimulating agents. This report discusses selected platelet secretory factors, including; IL-1 β , TGF- β , TGF- α , FGF, EGF, PDGF, and IGF, which support the concept that platelet concentrates can mediate cardiac wound healing. In conclusion, application of platelet gel to areas of cardiac injury may offer a therapeutic means to stimulate myocyte regeneration, angiogenesis, and restoration of a normal extracellular matrix composition. **Keywords:** remodeling, growth factors, fibroblasts. *JECT. 2004;36:191–196*

The development of platelet gel as a therapeutic agent has shown efficacy in supporting bone grafts, enhancing fracture healing, and accelerating wound healing (1–4). Platelet gel is composed of concentrated platelets that have been activated by numerous initiators including thrombin. Thrombin activation has been used to stimulate platelet aggregation and the release of stored growth factors from granules, forming an adhesive gel with a high concentration of factors required for wound healing. Application of this gel to wounds has shown marked increase in healing (4).

The acute and detrimental effect of a myocardial infarction is compounded by maladaptive remodeling induced by a dysfunctional healing process. The maladaptive healing process is caused by ischemia, aging, diabetes, and neurohormonal reactions. The consequence of maladaptive remodeling is a loss of normal cardiac ultrastructure and mechanical function caused by the overexpression of fetal genes caused by an inappropriate neurohormonal response (5). We have demonstrated that applying a fibroblast patch graft onto the epicardial surface of a cardiac infarction, normal adaptive remodeling can be achieved. This epicardial patch technique resulted in full and normal transmural remodeling, angiogenesis, restoration of nor-

mal calcium cycling protein expression, and restoration of extracellular matrix composition and function (6). However, because of the time required to isolate autologous fibroblasts, insert and grow the cells into a three-dimensional scaffold, and apply the patch to a site of infarction—an alternate means to provide appropriate cytokines, growth factors, and chemokines is being investigated. Table 1 shows that activated platelets secrete many of the factors that are secreted by fibroblasts, and it follows, therefore, that the application of autologous platelets may achieve similar efficacy demonstrated with the fibroblast patch.

PLATELET GEL

There are several methods for preparing platelet gel; however, most include the isolation and centrifugation of whole blood to isolate platelets. Addition of a solution of bovine thrombin has been used to activate the platelets. Calcium chloride is added to counteract the anticoagulant citrate, facilitate granule release and metabolism of arachadonic acid to thromboxane—a potent platelet activator. Upon the addition of the thrombin/calcium chloride solution, a thick, adhesive gel is formed. The thickness and adhesive nature of the gel is attributable to the fibrin platelet interaction. The adhesive nature of the gel is advantageous when applying it to a wound. When trying to supplement a wound with purified growth factors, the

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Table 1. Comparison of fibroblast and platelet secretory growth factors.

Secreted Factors	Fibroblast	Ref	Platelet	Ref
Cytokines				
IL-1 β	+	(39)	+	(40)
IL-10	+	(39)	-	(41)
IL-6	+	(39)	+	(42)
TNF- α	+	(39)	+	(42)
Growth factors				
Angiopoitin-1	+	(43)	+	(44)
bFGF	+	(39)	+	(44)
CSF-1	+	(39)	(-)	ND
EGF	-	ND	+	(44)
GM-CSF	+	(39)	(-)	ND
HGF	+	(39)	+	(44)
IGF-I and II	+	(39)	+	(44)
KGF	+	(39)	(-)	ND
NGF	+	(39)	(-)	ND
PDGF AA and BB	+	(39)	+	(44)
SCF	+	(39)	(-)	ND
TGF- β 1,2,3	+	(39)	+	(44)
VEGF	+	(45)	+	(44)
Chemokines				
ENA-78 (CXCL5)	+	(39)	+	(46)
GRO-1 α (CXCL1)	+	(39)	-	(41)
IP-10 (CXCL10)	+	(41)	-	(41)
IL-8 (CXCL8)	+	(39)	+	(46)
MCP-1 (CCL2)	+	(39)	-	(47)
MCP-2 (CCL8)	+	(47)	-	(47)
MCP-3 (CCL7)	+	(48)	+	(46)
MIP-1 α (CCL3)	+	(39)	+	(46)
NAP-2 (CXCL7)	-	(47)	+	(47)
PF4 (CXCL4)	-	(47)	+	(46)
RANTES (CCL5)	+	(39)	+	(46)
Inflammatory mediators				
PAF	+	(39)	-	ND
PGE ₂	+	(39)	+	(49)
Phospholipase A ₂	+	(39)	-	(49)
Prostacyclin	+	(39)	-	(49)
Others				
ACE	+	(50)	(-)	ND

method of delivery is a limiting and difficult problem (7,8). Interaction with synthetic adhesives can diminish the healing effect of the growth factor being delivered. Platelet gel naturally conforms to wounds, concentrating hyperphysiologic levels of growth factors to the site of injury, facilitating healing.

The preparation method of platelet gel can influence the concentration of growth factors released at the site of the wound. Purification of platelets is a complicated process, involving blood isolation, centrifugation, and extraction of platelet-rich plasma (PRP). If platelets become activated during the preparation phases, growth factor concentrations can diminish. There is disagreement about the stability of platelets and growth factors in stored samples. Zimmermann et al. reported that growth factors are released from platelets when stored from zero to 5 days, and the growth factors are not stable in plasma at 22°C (9). However, these results indicate platelet gel provides temporary hyperphysiologic levels of growth factors that can initiate the healing process.

Effects in Bone

Application of platelet gel at the site of bone grafts or surrounding fractures has improved healing. Previous studies have shown that recombinant FGF-2, a platelet gel growth factor, is able to accelerate fracture healing in tibial fractures of dogs, showing increased ossification and increased numbers of chondrocytes and osteoblasts (7). The use of platelet gel as an adhesive has become more widespread with bone graft procedures (10). PRP has been shown to promote rapid healing and revascularization of autologous bone graft in severely atrophic mandibles in humans (1). During the healing period of bone graft, proliferation and differentiation of osteoblasts is dependent upon adequate blood supply (3). The blood supply is extremely diminished in residual atrophic bone, the site of these grafts. Application of protein-rich plasma has shown revascularization of the implant site, making graft placement possible (4).

Effects in Skin Wounds

Platelet gel has also shown increased healing of soft tissue. The PDGF-BB isoform, a platelet gel growth factor, has been shown to promote the acceleration of healing in advanced pressure sores. Use of a topically applied 0.01% recombinant human PDGF gel significantly accelerates the healing of separated surgical wounds from an average of 54 to 35 days (8). Platelet gel has been demonstrated to promote total healing of severe ankle ulceration within 5 months of daily application of autologous platelet gel (11). The clinical experience in bone and skin wound healing support the concept that platelet gel could facilitate normal healing of the injured or diseased cardiac tissue.

GROWTH FACTORS

The benefits of platelet gel are derived from the synergistic effects of multiple growth factors. Activated platelets release many factors involved in wound healing, including platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), transforming growth factor-beta (TGF- β), epithelial growth factor (EGF), fibroblast growth factor (FGF), and interleukin-1 beta (IL-1 β). It is likely that these platelet-derived cytokines are involved with the early stages of wound healing (10). The platelet secretory factors are derived mainly from the megakaryocyte (12). However, there is cytokine and growth factor mRNA in the mature platelet, suggesting that mature platelets may have a limited ability to synthesize and factors before activation and degranulation (12). Understandably, once platelets are activated and degranulate, they have minimal biosynthetic ability to synthesize secretory factors in comparison to immune and fibroblast cells. Therefore, the efficacy of the reported platelet gels in

wound healing may reside in a “trigger effect” that creates an optimal microenvironment that initiates a normal healing process through facilitation and regulation of other cells, including the fibroblast.

PDGF

PDGF is one of the major growth factors released from the α -granules of activated platelets. There are three isoforms, homodimers, and heterodimers of the A and B polypeptide chains, designated PDGF-AA, -BB, or -AB. These isoforms activate two structurally related tyrosine kinase receptors designated the PDGF- α and - β receptors (13). Activation of the PDGF receptor activates cellular proliferation and migration of fibroblasts and promotes angiogenesis. PDGF has been shown to stimulate the synthesis of collagen and fibronectin and secretion of collagenases by fibroblasts (13). Deleting the genes in mice for the PDGF- β receptor shows defects in the development of blood vessels, dilated aorta (13), cardiac muscle hypotrophy, and widespread edema and hemorrhage (14). PDGF regulates the synthesis of its own receptor and also influences the expression of membrane receptors for IL-1 β , EGF, transferrin, and induces the expression of many new genes in numerous cell types. Therefore, the PDGF pathway relates the extracellular matrix composition, angiogenesis, and modulates other cellular functions.

IGF-1, -2

Insulin-like growth factor-1 and -2 are small peptide hormones that stimulate mitogenesis, promote differentiation, and inhibit apoptosis in many organs, including cardiac myocytes (15). IGF-2 has long been considered to be the fetal form of IGF-1 and important for embryo development. Normal activation of IGF-1 is essential for myocardial function, and IGF-1 deficiency is associated with impaired cardiac function. Acute administration of recombinant IGF-1 in patients with chronic heart failure improved cardiac function by exerting positive inotropic effects and afterload reduction (16). IGF-1 exerts receptor-mediated, Ca^{2+} -dependent positive inotropic effects by activating L-type Ca^{2+} channels (17). The described activity of this growth factor in healthy cardiac function supports its value as a component of platelet gel.

TGF- β

Transforming growth factor (TGF- β) is secreted by the platelet in μM concentrations compared to pM or nM with most other platelet-derived cytokines and growth factors. This high level of secretion underscores its critical role in wound healing. There are three mammalian isoforms: TGF- β 1, TGF- β 2, and TGF- β 3, which bind receptors

TBRI, II, and III. High levels of TGF- β receptors have also been found on fibroblasts and cardiac myocytes (18). TGF- β is a potent chemoattractant for fibroblasts and promotes proliferation, differentiation, and extracellular matrix synthesis. TGF- β is released from inflammatory cells, injured myocytes, and fibroblasts (19). It is a strong chemoattractant for monocytes and neutrophils; it stimulates wound closure and stimulates collagen synthesis (18). TGF- β 1 has been shown to stimulate the deposition of extracellular matrix into soft tissue and bone (18). TGF- β 1 mediated overexpression of collagen type I synthesis may be a major contributor to maladaptive remodeling of the myocardium and a contributor to vascular hypertension (20,21). However, TGF- β 1 is necessary for the deposition of new extracellular matrix proteins that serve as a substrate for new cardiac myocytes.

FGF

The fibroblast growth factors (FGF) include nine polypeptides that are known to play a role in angiogenesis and mitogenesis of many cells types. The most abundant FGFs are acidic FGF or FGF-1 and basic FGF or FGF-2 (22). Basic fibroblast growth factor has been shown to stimulate mitogenesis, angiogenesis, chemotaxis, and differentiation (23). It has been shown to have a vital role in the development of vascular, nervous, and skeletal systems and stimulates wound healing and tissue repair (23). Basic fibroblast growth factor (bFGF) itself has been shown to decrease myocardial infarct size in rabbits and stimulate angiogenesis of collateral vessels in infarcted areas (24). The defined role of FGF in wound healing is to stimulate healing of bone and modulate proper function of cardiac tissues.

IL-1 β , EGF, TGF- α

IL-1 β , EGF, and TGF- α are mediators of wound healing and inflammation. Interleukin 1 beta (IL-1 β) is important in wound healing, by stimulating fibroblast and keratinocyte growth as well as collagen synthesis (25). IL-1 β activates neutrophils, upregulates adhesion molecules, and promotes chemotaxis (25). The IL-1 β in platelet gel acts to increase the localized inflammatory process around the wound. Epidermal growth factor (EGF) is a secreted growth factor that stimulates fibroblast collagenase secretion and has a defined importance in wound remodeling. Transforming growth factor-alpha (TGF- α), a relative of EGF, has been shown to upregulate the formation of extracellular matrix and integrin expression, as well as to promote cell adhesion and motility (26).

Remodeling of the Myocardium after Infarction

Acute myocardial infarction (MI) results in the rapid loss of myocytes in the infarcted area of the heart. Necrosis and apoptosis of cardiac myocytes lead to remodeling of the myocardium. The inflammatory response following MI triggers the migration of platelets, neutrophils, macrophages, monocytes, and other inflammatory mediators to the infarct site, where secretion of matrix metalloproteinase (MMP) degrade components of the extracellular matrix. As myocytes are lost, regions of fibrosis are formed as collagen is deposited to replace dead myocytes (27). The fibrotic areas of infarction, of course, lack contractile ability and without regional myocyte regeneration, extracellular matrix formation, and angiogenesis, the wall stress increases leading to maladaptive remodeling.

The recent discovery of cardiac myocyte proliferation in areas adjacent to infarcted areas of the heart has shown that it is possible for the myocytes to regenerate. Beltrami et al. examined the expression of Ki-67, a nuclear protein only expressed in dividing cells, from areas surrounding infarcted zones of the heart. Ki-67 expression was detected in 4% of the myocyte nuclei in regions adjacent to the infarcts and 1% of those in regions distant to the infarcts (28). This study implies that regeneration in the myocardium may be possible. It is still undetermined if these proliferating myocytes are dividing cardiac myocytes, resident stem cells, endothelial cells, fibroblasts, or adult hematopoietic stem cells (HSC) that have migrated into cardiac tissue.

There is evidence that the plasticity of adult stem cells is sufficient to engraft into the heart. The plasticity of HSCs has been well documented with adult-derived bone marrow stem cells differentiating into neural (29,30), skeletal muscle (31,32), and hepatic cells (33). It is established that there are endothelial progenitors that do serve as stem cells for angiogenesis, which opens the possibility that endothelial progenitors may serve as cardiac myocytes (34). Experimental studies in cell culture and animal models have demonstrated the potential for various types of progenitor cells, including embryonic stem cells and hematopoietic cells, to differentiate into functional cardiac myocytes with the ability to repair damaged myocardium (35). Injection of fluorescence-labeled HSC in the border zone subsequent to a coronary ligation has been shown that HSC differentiate into proliferating myocytes and vascular structures (36). Established fluorescence-labeled HSC have been shown to migrate to the ischemic/reperfused myocardium and to differentiate into myocytes and endothelial cells (37). The adult dystrophic mdx mouse model demonstrated recruitment of bone marrow-derived cells that underwent myogenic differentiation (38). Human studies that examined post-mortem or cardiac explant myocyte proliferative activities demonstrated markedly increased myocyte proliferative activities that represented

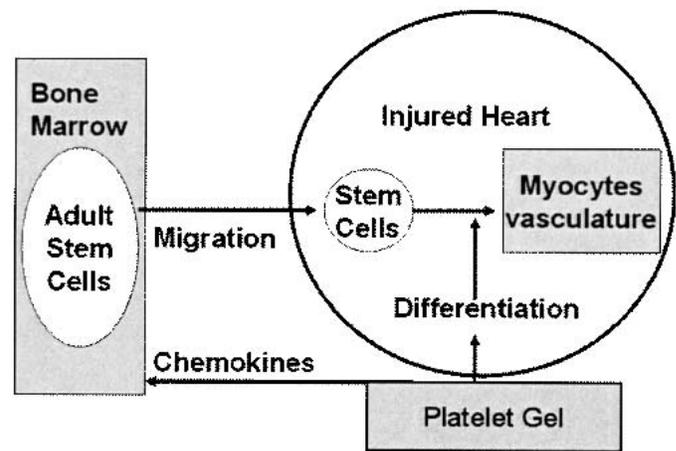


Figure 1. Proposed mechanism of cardiac regeneration by platelet gel.

proliferation of diseased tissue at a single time point (15,28). Because of the apparent plasticity of adult stem cells, it is becoming clear that the potential source of the stem cells may not be as important as the microenvironment that supports the stem cell engraftment. Therefore, the possibility exists that platelet gel may provide or trigger the supportive microenvironment to facilitate adult stem-mediated myocyte regeneration, as diagrammed in Figure 1.

The maladaptive remodeling of the myocardium after infarction eliminates the support structure, and extracellular matrix for myocytes. Fibrotic lesions do not contain the necessary vasculature or growth factors to support the growth of new myocytes. The application of platelet gel to the epicardium during bypass surgery or to internal vessels in the cardiac catheterization laboratory has the potential to provide myocardial regeneration. The concentrated platelet growth factors may stimulate myocyte recruitment and differentiation of stem cells to the infarcted areas. TGF- β supports the formation of the extracellular matrix and the secretion of other cytokines. PDGF and bFGF have been shown to stimulate angiogenesis. Formation of new blood vessels into fibrotic tissue is a necessary step for myocardial regeneration. Platelet gel contains chemokines that could support stem cell recruitment and differentiation (see Table 1). Finally, the adhesive nature of the gel would make it possible to concentrate these growth factors over the infarcted areas.

CONCLUSION

Given the profound impact platelet gel has had on improving healing in bone graft and tissue injury, this technique needs to be explored as a possible means of regeneration of the myocardium. Manipulating the body's response to injury as a means to regenerate tissue could have a profound impact on heart disease. The isolation and activation of platelets is a simple, safe procedure and an

established technique. The application of the gel could be performed during surgery and with more effort in the cardiac catheterization laboratory. The application of platelet gel to the injured myocardium offers the possibility of preventing or reversing maladaptive remodeling. Future studies will determine the efficacy of platelet gel in the treatment of heart disease.

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