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The Action of Platelet-Rich Plasma (PRP) in Cardiovascular Disease Treatment

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ABSTRACT

Atherosclerosis, or coronary artery disease, is an inflammatory disorder capable of affecting large arteries. It is also the main cause of cardiovascular disease and stroke, and its main causative factors lie on lipid accumulation in, and inflammation of, large arteries. The aim of the current study is to investigate the need of conducting better therapeutic assessments in more clinically relevant animal models of ischemic stroke, as well as the action of platelet rich plasma in modulating inflammatory processes in cardiac injury cases.

Introduction

The Brazilian Society of Cardiology (SBC – *Sociedade Brasileira de Cardiologia*) issued an alert in the World Heart Day, celebrated on September 29. According to the aforementioned entity, Brazil was expected to register approximately 400,000 deaths associated with cardiovascular diseases by the end of 2022 [1].

More than 289 thousand people in Brazil died of cardiovascular diseases in 2019, based on the Cardiometer platform of the Brazilian Society of Cardiology [2].

According to the World Health Organization (WHO), cardiovascular disorders are the main cause of deaths, worldwide. According to SBC, heart diseases lead to twice as many deaths as the combination of all cancer types, in Brazil [1].

Atherosclerosis, or coronary artery disease (CAD), is an inflammatory disorder capable of affecting large arteries. It is also the main cause of cardiovascular disease (CVD) and stroke, and its main causative factors lie on lipid accumulation in, and inflammation of, large arteries [3-7].

Figure 1 shows endothelial cells (ECs) that generate a single cell layer bound by firm bindings separating the blood from the vessel wall [3]. ECs, and their tight junctions, suffer with a leak-

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like process under disturbed blood flow conditions; this process stimulates plasma low-density lipoprotein (LDL) and TG-rich lipoprotein (triglyceryl-lipoproteins) absorption by trans-endothelial derivatives or through diffusion at cell-cell combinations [8]. ECs activation takes place in response to the oxidation of lipoproteins, lipids and other inflammatory transitional cells [9,10]. It emerges in P-selectin, VACM1, ICAM1 and E-selectin expression, which leads to the production of monocytes, different leukocyte types and chemotactic factors, such as CCR5 and CCR2 [11].

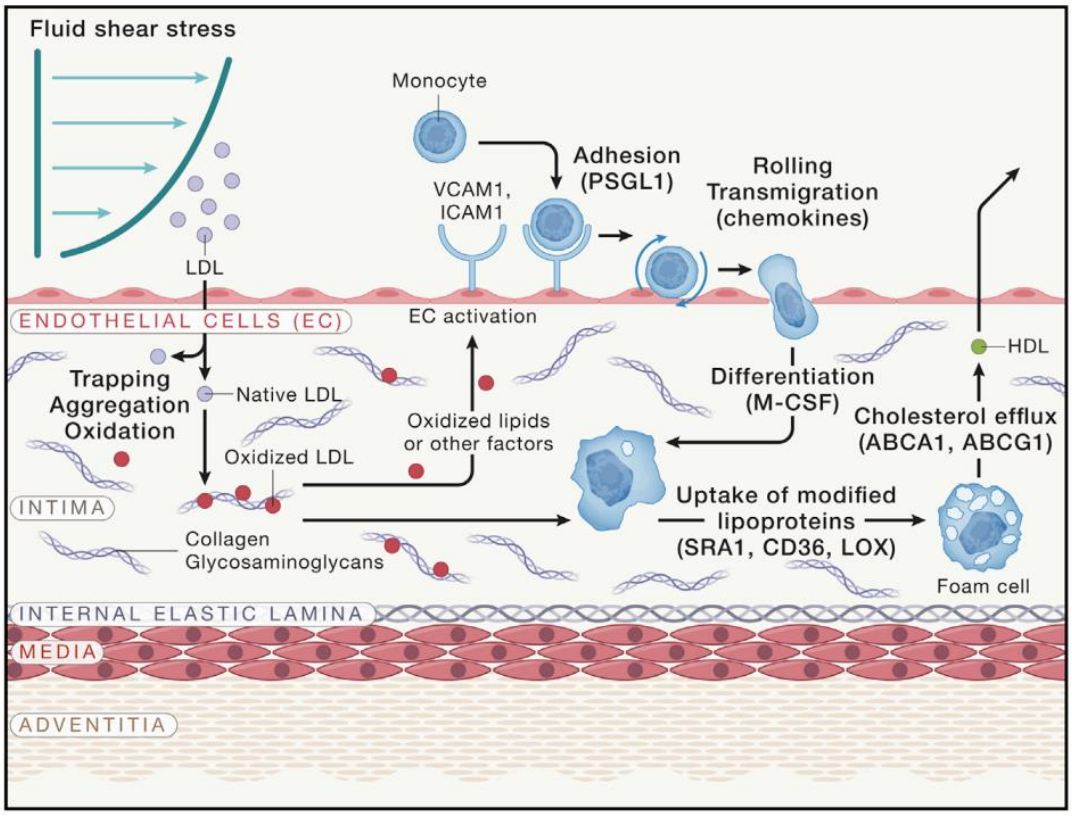

Figure 1: *Development of fatty streak lesions. Lipoproteins enter the intima at of sites of shear stress. The lipoproteins then aggregate and become oxidized and otherwise modified, resulting in the activation of the overlying endothelial cells (EC) to express adhesion and chemotactic molecules for monocytes. The monocytes enter the intima, differentiate to macrophages, and take up modified lipoproteins to give rise to foam cells. The size of the intima is exaggerated in the figure (from reference [3]. Permission under the CC BY license (http://creativecommons.org/licenses/by/4.0/) From Cell Press, Elsevier Inc.).*

Processes like disturbed blood flow can cause plaque erosion, which is an optional EC dysfunction pathway (Fig.2). TLR2-dependent EC apoptosis is also caused by IL-8 release, which leads to neutrophil enlistment, as well as to the activation and liberation of neutrophil extracellular traps that worsen the damage to the EC layer and likely lead to thrombus emergence, under this condition [12].

Shear stress induces characteristic EC alignment in atherosclerosis [13,14], for example (Figure 2A). It is important to see wherever the elongation and WSS (wall shear stress) present laminar flow in arteries' straight regions or tubular section, and ECs show flattened shape and elongated alignment in the flow direction [15]. However, disturbed flow happens either at the bifurcation or elevated vessel curvature site and ECs increase their volume by embodying a

cobblestone shape due to agitated and regressive flow with low WSS at the external vessel wall [16] (Figure 2A). Furthermore, hemodynamic strength defines the early advancement of localized atherosclerotic plaques that are not accidentally dispersed in murine or human models [17,18]. Atherosclerotic wounds mostly happen in regions differentiated by low WSS and flow separation (Figure 2B); almost all of them often present branch points and bifurcations. Significant correlation observed in data showing that low shear or increased flow explains the position of atherosclerosis injuries has emphasized the significant part played by arterial ramifications and bifurcations at the time to diagnose atherosclerotic damage progress or advancement [19].

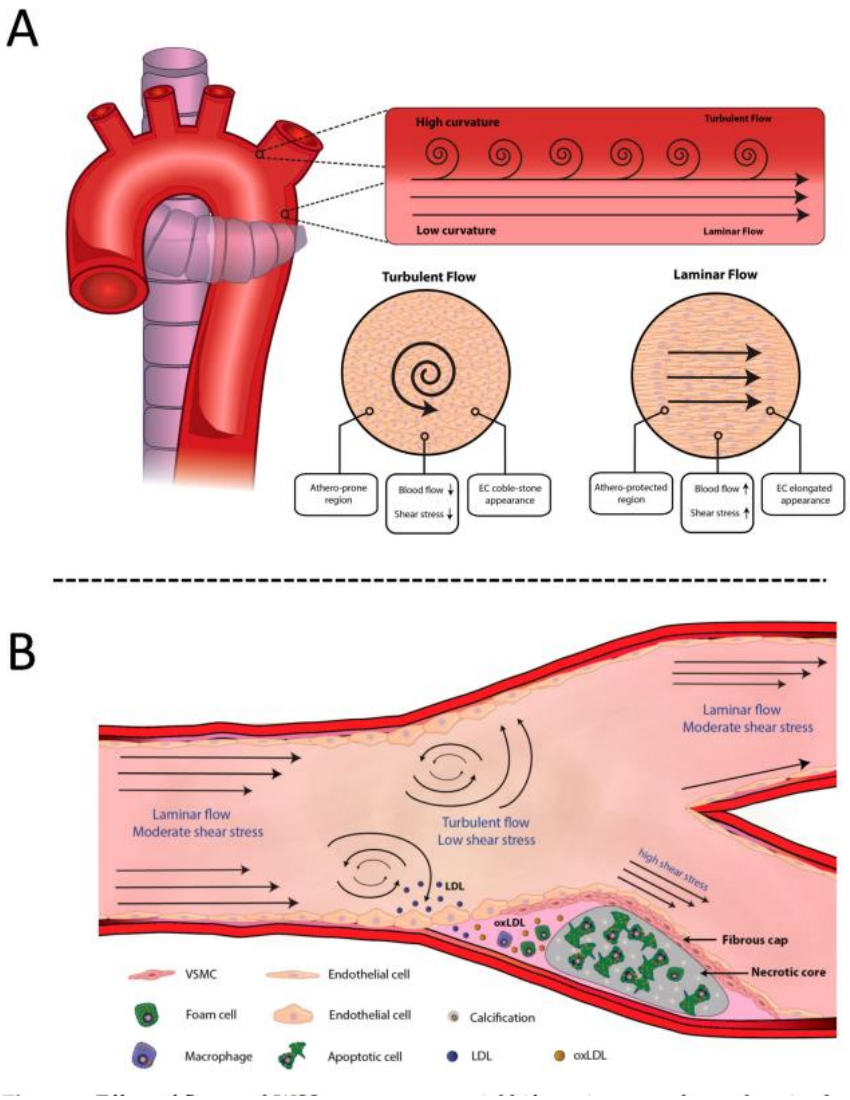

Figure 2: *Effect of flow and WSS patterns at arterial bifurcations on atherosclerotic plaque development. (A) In straight vessel segments, physiological WSS with laminar flow leads to ECs and shows a quiescent characteristic flattened shape when flow disturbance occurs. Lower WSS at the outer vessel wall causes ECs to adopt a cobblestone appearance. (B) Turbulent flow occurs at bifurcations and branch points where the arterial curvature is higher due to flow separation. Disturbed laminar flow or turbulent flow reduces WSS and promotes endothelial dysfunction and LDL infiltration, which constitutes the first step of atheroma plaque formation. On the contrary, low curvature areas of the vascular system subjected to higher shear stress are athero-protected (from ref. [19], under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0)*

Animal models used to investigate atherosclerosis or atherosclerosis-related diseases

Recent study has shown that PRP can cause mesenchymal stem cell (AMSCs) differentiation in cardiomyocytes by releasing growth factors, such as PDGF, IGF, FGF and TGF-β. It has found difference in AMSCs contrast in cardiomyocytes after PRP application, in comparison to α-MEM and Cardiomyogenic Kit media. These aspects were evidenced by GATA-4 expression at the 5^{th} day after PRP application and by Troponin (cTnT) expression at the 10^{th} day after PRP application. Therefore, PRP – which is simple, easy to obtain and has lower cost – has significantly enhanced AMSC differentiation in cardiomyocytes in research conducted *in vitro* [20].

Important criteria adopted to select animal models comprise size, docility, easy breeding and housing, well-known genetic data, being the nearest simile to humans and costs associated with all these parameters. Overall, small murine (e.g., mouse) and rabbit models provide important information about atherosclerosis pathophysiology and etiology. On the other hand, bigger animal models, such as porcine and monkeys, are more reliable and closer to humans when it comes to diseases [21,22]. These models play important part in some procedures, such as imaging methods and processes focused on estimating the efficiency of pharmacological procedures. With respect to nowadays knowledge about genetic techniques, the progress achieved with mini pigs provided an adequate human-like physiology alternative to monkeys. In addition, this animal model requires simpler handling than mice or rats, as well as presents higher similarity to human cardiac structure, physiology, lipid metabolism and atherosclerotic pathophysiology. Thus, it is expected to behave as relevant model in studies conducted *in vivo* aimed at improving sensitive biomarkers and authenticated imaging tools used to predict plaque rupture, which is the main clinical condition leading to death cases in patients with atherosclerosis [23].

As previously mentioned, several safe mice and rabbit models are well known and validated for research on atherosclerosis. Overall, almost all studies conducted in this field are based on genetic mutations of specific genes working in atherosclerosis progress, such as apolipoprotein E or LDL receptor genes. These animal models differ from each other in terms of blood lipid outline, ability to heal from atherosclerotic lesions (either the spontaneous ones or those caused by a given diet) and emergence of complex and unstable plaques. Although atherosclerosis lesions can be safely induced in animal models, modeling complex plaques with aspects, such as neovascularization, calcification, intraplaque hemorrhage and thrombosis, is more demanding. Then, it is of paramount importance conducting studies focused on investigating animal models that can be used to assess new treatments aimed at plaque stabilization processes [24].

Different animal models have been widely used to analyze the pathophysiology of cardiovascular disorders or ischemic heart diseases. Table 1 [25] describes the model used to investigate the PRP effect on ischemic heart disease cases.

It is important emphasizing that promising treatments have been developed based on using animal models. Although most of them presented effective outcomes in tests conducted with rodent models, almost all interventions have failed in clinical trials conducted with humans. This negative preclinical-to-clinical translation outcome highlights the crucial need of improving therapeutic assessments in more clinically relevant animal models of ischemic

stroke [26]. Bigger animal models, such as pigs, sheep, dogs and non-human primates, are likely more predictive of human responses and outcomes since their brain anatomy and physiology are quite similar to that of humans; this similarity potentially turn bigger animal assays into key step in the stroke therapy translational pipeline [27].

Table 1: *Some examples of the effects of platelet-rich plasma in ischemic heart disease (Modified from Ref. [25]).*

Ref.	Type of study	Animal model	Delivery method	Effect
[33]	Experimental	Sheep	Implantation	Increased formation of new vessels
[34]	Experimental	Rabbit	Intramyocardial injection	Reduced reactive oxygen species generation Stabilized the mitochondria of the ischemic/reperfused heart
[35]	Experimental	Mice	Intramyocardial injection	Higher left ventricular ejection fraction after ischemia
[36]	Experimental	Porcine	Intramyocardial injection	Attenuated adverse cardiac remodeling
[37]	Experimental	Rats	Intramyocardial injection	Decreased infarct size Increased ventricular wall thickness Improved cardiac function and reperfusion
[38]	Experimental	Rat	Intramyocardial injection	Limitation of ventricular expansion, Attenuated myocardial hypertrophy in the noninfarct region Facilitated angiogenesis and arteriogenesis in the infarct
[39]	Experimental	Rats	Intramyocardial injection	Improved LV performance
[40]	Clinical	-	Intramyocardial injection	More efficacious at relieving angina Improved myocardial function
[41]	Experimental	Mice	Ischemia-reperfusion (I/R) injury	Enhanced the survival area and perfusion of the flap, reduced neutrophil accumulation in mice subjected to I/R injury

Platelet-rich plasma (PRP)

Platelet-rich plasma (PRP) is defined as autologous blood fraction presenting large amounts of different growth factors. Among them, one finds EGF (epidermal growth factor), VEGF (vascular endothelial growth factor), HGF (hepatocyte growth factor), TGF-β (transforming growth factor beta-1), FGFs (fibroblast growth factors), IGF-I (insulin-like growth factor-I) and PDGF (platelet-derived growth factor), which have the potential to be used in repair and regeneration processes [28-32].

PRP And Atherosclerosis

Endothelial progenitor cells (EPCs) play key part in regulating vascular wall integrity and homeostasis in order to avoid thrombosis and vessel inflammation, since these conditions can induce coronary artery disease.

Growth factors appear to play the most important part in stimulating transduction signal in EPC proliferation procedures. The total blood component called PRP presents several growth factor types that are well known for playing key part in homeostasis and healing processes.

Different growth factors observed in PRP have different functions in angiogenesis and blood flow repair processes taking place after ischemic events. It is consensus that VEGF is the main post-ischemia angiogenesis inducer factor [42] since it induces endothelial cell proliferation and relocation to enable angiogenesis. PRP induction in angiogenesis processes is controlled through PI3 kinase and extracellular signal-regulated kinase (ERK) stimulation [43]. However, neovascularization is a complex event, since the interaction between cells and angiogenic factors other than VEGF requires the generation of stable capillary vessels [44].

Thus, experimental mononuclear cells were collected *in vitro*, extracted from peripheral blood samples of patients with Stable Coronary Artery Disease (SCAD) and cultured in proper M-19 medium [45]. Immunocytochemical analysis was carried out after 14-day culture. EPCs were marked with CD34 and cell-counting results have shown significantly increased EPC proliferation in the PRP group (1.052) in comparison to that of the control group (0.068). These results have evidenced that PRP has effectively increased EPC proliferation in comparison to the control group, as well as that it avoided thrombosis and vessel inflammation.

Bir et al. [46] have also shown the effectiveness of sustained PRP release in stimulating therapeutic neovascularization processes, and it led to angiogenesis, arteriogenesis and vasculogenesis in murine models with hind limb ischemia.

Da Silva et al. [47] investigated the effect of human platelet-rich plasma (PRP) on atherosclerosis progress in knockout mice, based on using LDLr -/- (low-density lipoprotein receptor). The mice model with atherosclerosis was assessed based on several parameters, such as weight, cholesterol and triglycerides serum levels, TGF-β (transformer-β growth factor) levels and different cytokine types. All parameters associated with atherosclerosis progress in mice recorded abnormal results: cholesterol levels reached 172.0 vs. 249.5 mg / dL, whereas triglycerides levels reached 58.6 vs. 110.0 mg / dL, in normal diet or HF-fed mice, respectively. Parameter "HF-fed animals' weight" was also measured; it recorded 25.5g, whereas the normal diet-fed group recorded 18.5 g. Triglycerides levels in this group decreased in comparison to the control, after 1-month PRP application. However, cholesterol levels in this group were almost identical to those of the control group, after 2-month PRP application. The opposite

result was observed for triglyceride levels, which decreased in the serum group in comparison to the PRP group. Models' pro-inflammatory profile has shown decreased, although non-significant, cytokine serum levels after 1-month PRP application; it was followed by decrease in TNF-α, IFN-γ, IL-12 and IL-17 levels. Although non-significant, these data have indicated improvement in models' inflammatory condition due to increase in IL-10 and IL-13 levels. The aforementioned authors have suggested that lyophilized PRP application in the investigated atherosclerosis model provided short-term protection against this disease, as evidenced by reduced cholesterol and triglycerides levels, as well as by enhanced outline of pro- and anti-inflammatory cytokines.

Platelet-membrane-coated mesoporous silica nanoparticles (PMSN) were recently reported as programmed releasing system for specific sites, since it delivers antiCD47 antibody to atherosclerotic sites. PMSN was capable of mimicking the platelet in order to evade immunoclearance in the reticuloendothelial system and to specifically target atherosclerotic plaques, pile up in the necrotic cores, and liberate the antibody. PMSN has escaped macrophage recognition *in vitro*, as well as presented long circulating half-life *in vivo,* in comparison to MSN (non-modified mesoporous silicon nanoparticles). PMSN has mainly accumulated in atherosclerotic plaques of apolipoprotein E–deficient (apoE−/−) mice (animals prone to show spontaneously increased atherosclerotic lesions under standard chow diet) with atherosclerosis. CD47@PMSN nanoparticle has effectively avoided cells' necrotic decline in comparison to the free anti-CD47 antibody; most importantly, it led to efferocytosis of necrotic cells in the plaques. Dead cells have significantly diminished atherosclerotic plaque surface, stabilized plaques, as well as mitigated the risk of plaque rupture and progressed thrombosis. PMSN was totally safe for the investigated animals. Moreover, membrane-coated MSN (PMSN) loading anti-CD47 antibody was useful in the healing approach applied to atherosclerosis [48].

Role Played By PRP In Modulating Inflammatory Activity In Cardiac Injuries

One of the most important activities performed by PRP lies on inducing tissue turnover by releasing several growth factors from α-granules into platelets. Primary PRP treatment goals lie on stopping the actual inflammatory and catabolic microenvironment, as well as on inducing initial heart damage repair and regeneration processes. This mechanism is known as "regenerative inflammation" [49]. Studies have shown that thrombin PRP stimulation promoted biomolecular liberations [50]. Moreover, this process releases growth factors, such as HGF, EGF, TNF-α and TGF-β1. Besides all these processes, PRP stimulates VEGF type-II collagen and aggrecan mRNA responses, such as reducing their limitation or suppression by pro-inflammatory cytokine interleukin β1 [51]. HGF appears to play important part in PRP's anti-inflammatory activity. Anti-inflammatory cytokines mitigate inflammation processes by inhibiting NF-κB signaling mechanism [52]. Cytokines also control inflammation processes by interacting with soluble cytokine receptors and suppressors. IL-1 receptor antagonists - IL-n (n = 4, 10, 11 and 13) - are major anti-inflammatory cytokines. Other cytokines, such as IFN- α (interferon-alpha), TGF-β1, IL-6 and leukemia inhibitory factor perform pro- or anti-inflammatory actions, depending on wound type. Receptor cytokines, such as TNF-α IL-1 and IL-18, can suppress other proteins with pro-inflammatory properties [53]. Significantly active anti-inflammatory cytokines, such as IL-10, produce pro-inflammatory cytokines, such as

TNF-α, L1 and IL-6, while increasing the production of anti-inflammatory factors [54]. It is important emphasizing that these negative aspects of feedback systems do not play significant part in the generation of, and part played by, pro-inflammatory cytokines [55].

In addition, specific cytokines are capable of activating different signaling pathways to boost fibroblasts that, in their turn, play critical part in tissue healing processes [53]. Pro-inflammatory cytokines, such as IL-n (n = 1, 6, 13, 33) and TGFβ1, enable fibroblast differentiation into myofibroblast and help improving extracellular matrix (ECM) [54]. Several chemokines (e.g., TGF, IL-1, IL-33, TGF, CC and CXC) are successively released by fibroblasts to stimulate collections of immune cells like macrophages and to induce pro-inflammatory response [55].

All these inflammatory cells are required for tissue generation purposes [56]. VEGF, TGF-β and PDGF are pro-angiogenic stimulators that play important part in these regeneration processes. Researchers have evidenced that PDGF is capable of inducing EC differentiation as arteriogenic agent [57,58]. However, several growth factors, such as PDGF and TGF- β, affect the angiogenic potential of cells [59]; however, the last aforementioned effects were assessed based on using a mixed growth factors' solution. Interestingly, PDGF enables blood vessels to functionally expand within growth factors[60]. Accordingly, blood vessels would increase due to several prostaglandin F2-α metabolite and FGF liberations. PDGF helped enhancing the maturation of freshly formed blood vessels, either alone or in combination to VEGF liberation [61]. According to Lana et al. [62], fibrin wasting products likely worked as molecular intermediates, and it enabled tissue healing, as well as fibrin deposition and full removal, to trigger the angiogenesis process necessary for wound healing, as previously observed for fibrinolytic reactions. Thus, as previously mentioned, cytokines deriving from PRP play key part in inducing cell-mediated immunological response and in resolving the inflammatory phase (called regenerative inflammation) [48].

Actually, the inflammatory phase plays essential part in effectively enabling tissue healing, despite disturbing the patients, since it induces cell plasticity [63]. Hargrave and Li [64], reported increased contraction/relaxation rate in the left ventricle of models subjected to PRP treatment, as well as diminished infarct size during cardiac treatment in comparison to saline control. Mitochondrial depolarization and ROS (reactive oxygen species) production by PRP-treated cells have decreased. These outcomes are indicative that PRP helped protecting models' heart by stabilizing mitochondria and by reducing ROS production in the ischemic heart. Direct PRP injections in the myocardium helped improving ventricular use and myocardial infusion in rats [36]. Notably, the PRP group presented ejection fraction (EF) significantly higher than that of the control group, as well as effectively enhanced myocardial perfusion. PRP was effective in several parameters investigated in cardiac studies followed by histological monitoring, since it reduced infarct size, increased ventricular wall thickness and enhanced cardiac achievement [28]. Moreover, Li et al.[38] have shown that the platelet-based paracrine mechanism was capable of accelerating healing process of early-stage myocardial damage in mice. Ischemic myocardium treated with thrombin-activated PRP presented enhanced ventricular remodeling, as evidenced by easy neovascularization, ventricular length limitation, infarct arteriogenesis and non-infarct myocardial mitigation.

ADMSC (adipose-derived mesenchymal stem cells) in PRF (platelet-rich fibrin scaffold) have shown advanced ADMS-oriented outcomes, such as enhanced left ventricular (LV) activity, decreased LV remodeling and improved post-myocardial infarction model (MI) [29] (Fig. 3).

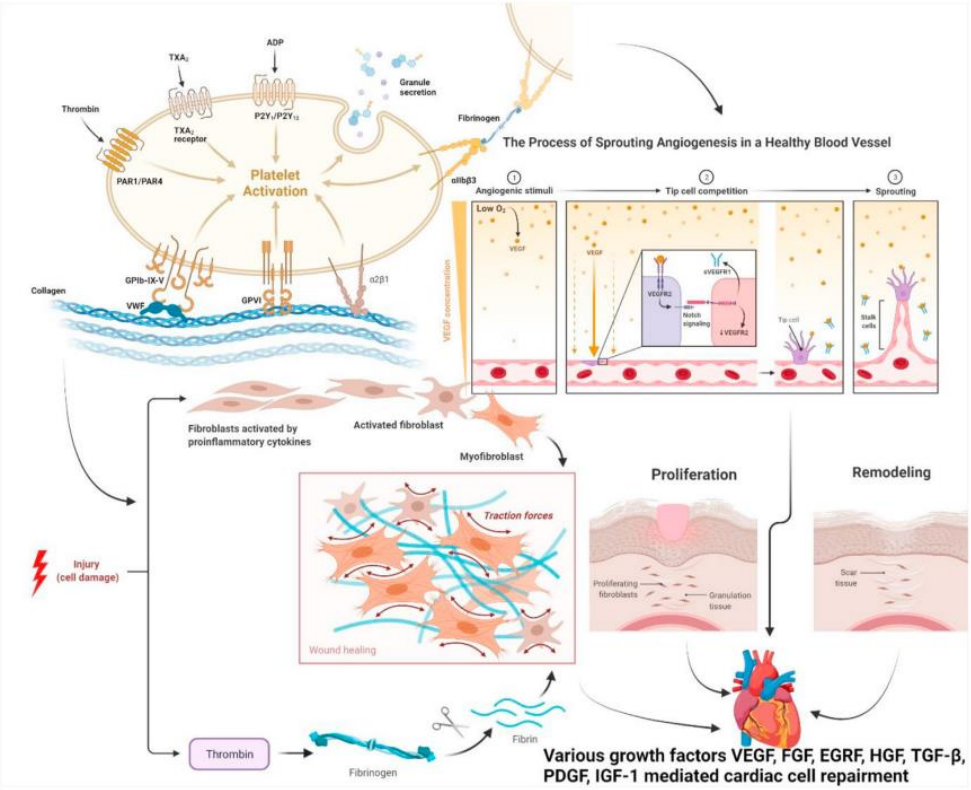

Figure. 3: *Platelet rich plasma mediated repair and regeneration of cell in early stage of cardiac injury (from Ref. [28], by permission of The Japanese Society for Regenerative Medicine. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)*

PRP effect on ischemic flaps was investigated based on using BLI (bioluminescence imaging) conducted *in vivo*; results were substantial enough to enable investigating angiogenesis in wounds. Calculations of VEGFR2 expression contents *in vivo* were also monitored. Results have evidenced that PRP application in ischemia cases had positive impact on angiogenesis and enabled full healing. The association of the two outcomes has significantly improved angiogenesis and flap survival rates, as evidenced in the bioluminescence imaging of VEGFR2 activity. Therefore, the Sönmez et al [64] suggested that local PRP application to the inner surface of a tissue plane after the ischemic period can lead to significantly improved flap and plane healing, as well as increase individuals' survival rate. Finally, these outcomes have indicated that PRP application in microsurgical free tissue transfers can likely mitigate deleterious effects of total ischemia [64]

Conclusion

Atherosclerosis, or coronary artery disease (CAD), is an inflammatory disease affecting large arteries. Overall, it is seen as the main causative factor of CVD (cardiovascular disease) and stroke. Rodents are the animal models mostly used to investigate this disease; however, based on studies available in the literature, it is clear that these assays fail in human clinical trials. Thus, it is recommended using more clinically relevant ischemic animal models for such a

purpose. The literature in this field suggests using larger animals, such as pigs, sheep and non-human primates, which are more likely to best represent human diseases. EPCs (endothelial progenitor cells) play key part in regulating vascular wall integrity and homeostasis, as well as in avoiding thrombosis and vessel inflammation, since both processes can induce CAD pathogenesis. Interestingly, growth factors play key part in stimulating signal transduction to EPCs and a total blood component named platelet -rich plasma (PRP) plays important role in this process. The autologous blood fraction known as PRP comprises a wide variety of growth factors, such as EGF, HGF, VEGF, FGFs, TGF-β, IGF-I and PDGF, which can favor repair and regeneration processes. Thus, assays conducted *in vitro* with samples collected from patients with SCAD have monitored the immunocytochemical outcomes of EPC marked with CD34. Results have evidenced significant increase in EPC proliferation after PRP application, which avoided thrombosis and vessel inflammation. Results in a study focused on investigating the effect of human platelet-rich plasma (PRP) on atherosclerosis progress in knockout mice, based on using the LDLr -/- (low-density lipoprotein receptor), have suggested that lyophilized PRP application in the atherosclerosis model had short-term protection effect, which was featured by reduced cholesterol and triglycerides levels and by improvement in the profile of pro- and anti-inflammatory cytokines. Moreover, ischemic myocardium treated with thrombin-activated PRP presented ventricular improvement, as evidenced by easy neovascularization, ventricular enlargement limitation, infarct arteriogenesis and non-infarct myocardial mitigation. Thus, PRP has evident effect on atherosclerosis and CVD cases, as well as significant potential to be used to treat these diseases.

Declarations

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Authors Contribution

ND: Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing - original draft. ACML: Data, collection and analyses. WJF, ACML: Formal analysis, supervision of manuscript. Final editing manuscript.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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