

UDC 616.74-003.93-085.382

Potentials and impact of platelet-rich plasma (PRP) on the regenerative properties of muscle tissue

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Background. Platelet-Rich Plasma (PRP) is often used in various fields of medicine. The current state of the art in the use of PRP holds great promise for the development of the methodology. PRP can improve the course of many diseases. In this article the impact of PRP on the regenerative properties of muscles is discussed. There are many protocols for preparing PRP, each with its own standardised parameters and claimed results. The article presents a review of the literature on the use of platelet-rich plasma, focusing on the definition of PRP, various methods of preparation and activation, as well as the concentration of growth factors.

Keywords: platelet-rich plasma, muscle regeneration, recovery, muscle injuries, tissue repair.

Abbreviations: PRP — platelet-rich plasma, FGF — fibroblast growth factor, EGF — epidermal growth factor, PLGF — placental growth factor, TGF- β — transforming growth factors beta, MSCs — mesenchymal stem cells

Introduction

The PRP use has become an increasingly popular method in the field of regenerative medicine in recent years [1]. PRP is a biologically active material obtained by enriching a patient's platelet sample with platelets. Platelets, while playing an important role in blood clotting, also have the potential to influence tissue regeneration. In recent years, PRP has been shown to possess a be-

neficial effect on the regenerative properties of muscles [2–4].

Muscle injuries are common in active lifestyles and sports [5, 6]. Fast and effective recovery of muscle tissue is a key for returning to normal body function and preventing complications [7]. The traditional treatments such as physiotherapy, massage, and analgesics can be effective but often take long time to full

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recover, unlike PRP therapy. PRP therapy is used in musculoskeletal and aesthetic medicine to support healing and regeneration [8]. Promising outcomes have been observed when employing PRP therapy, particularly with the Angel® system, in retrospectively assessing its efficacy among the individuals with a range of musculoskeletal disorders like knee osteoarthritis, lumbar degenerative disc disease, rotator cuff injuries, and other related conditions [9]. Unlike traditional treatments, PRP therapy has the potential to enhance recovery and improve outcomes [10].

PRP offers a new approach to muscle regeneration by utilizing the body's own biological resources [11]. PRP has demonstrated its effectiveness in enhancing muscle recovery through the mechanisms such as supporting the regeneration of muscle fibers, aiding in the restoration of electrophysiological function, and mitigating the formation of fibrotic tissue [3, 12]. PRP also boosts the function of satellite cells, crucial for muscle regeneration, by increasing the expression of myogenic regulatory factors [4, 13]. This method involves injecting platelet-rich plasma directly into the affected muscle area [14]. Platelets contain growth factors, biologically active substances that stimulate the tissue healing and promote the development of new cells and blood vessels [15, 16].

Results and Discussion

The research methods used to study the effect of platelet-rich plasma (PRP) on the muscle regeneration properties may vary depending on the specific research study [3, 17]. The most general approaches and methods that can be used in such studies are the next.

1. Patient selection: to study PRP and its effects on muscle regeneration, the patients with muscle injuries or damages that require regeneration are usually chosen. It is important to collect information about the patients' age, gender, medical history, and injury characteristics [4, 18].

2. Preparation of PRP: the patient blood samples are collected and subjected to a centrifugation procedure to separate the platelets from other blood components. This can be done using the commercial PRP systems or standardized protocols [19, 20].

3. Assessment of the platelet concentration: the platelet concentration in PRP is usually measured by laboratory methods using hematology analyzer or cell counter [21].

4. PRP application: the PRP is applied to the affected muscle area using an injection method or other techniques that may be appropriate for the particular study. Control groups may receive placebo or standard treatments [22].

5. Evaluation of the regenerative properties: various methods are used to evaluate the effectiveness of PRP. They can include clinical examination of patients, physical tests that assess the muscle strength and function, imaging studies (e.g., ultrasound) to determine structural changes in muscle tissue, biomarker analysis (e.g., growth factor content), and other methods used to assess the regenerative processes [23].

6. Statistical analysis: the data obtained are subjected to statistical analysis to determine the statistically significant effect of PRP on the regenerative properties of the muscles. This may include the calculation of mean values, standard deviation, t-tests, analysis of variance and other statistical methods.

PRP is an autologous concentration of platelets in the plasma with numerous growth factors that promote muscle regeneration and overall recovery. This method has gained popularity in the treatment of various muscle injuries due to its autologous nature, minimal invasiveness, lack of side effects, and greater availability and affordability compared to other methods. The mechanism of action of PRP in the treatment of various injuries is a complex multi-step process involving the regeneration factors and more effective muscle repair [3, 24].

The platelet physiology and role in regeneration

Platelets, also known as hematopoietic cells, play a key role in blood clotting and hemostasis. When a vessel is damaged, platelets are activated and aggregate to form a platelet clot. This process involves several sequential steps, including platelets adhesion to the damaged vessel, their activation and maturation, and secretion of clotting-promoting biomolecules, including clotting factors [15].

In addition to the role in blood clotting, platelets also have the properties that promote tissue regeneration. They contain many biologically active substances, including growth factors, cytokines, anti-inflammatory molecules, and other biomolecules. These substances play an important role in attracting and activating various cells involved in regeneration processes [16].

Additionally, platelets can stimulate the migration of cells, such as fibroblasts, endothelial cells, and myogenic precursors, to the site of injury. This promotes the formation of new tissue and regeneration of a damaged muscle fiber. Moreover, platelets can promote

angiogenesis, the development of new blood vessels that are necessary to supply nutrients and oxygen to the regenerating tissues [25].

One of the main factors contained in platelets that play an important role in tissue regeneration are growth factors. These biomolecules, such as FGF, EGF, PLGF and others, promote cell proliferation, differentiation, and synthesis of extracellular matrices [26].

These growth factors affect various cell types, including muscle cells, and promote their regeneration and repair. They activate the signaling pathways that control the cell proliferation and differentiation and promote the production of myoxin, an important component for the muscle tissue healing.

Measurement of the Platelet concentration in PRP

The platelet concentration in PRP is an important factor because it affects the effectiveness and results of the regenerative process. Typically, PRP contains a higher platelet concentration than normal blood. The platelet concentration in PRP can vary depending on the protocol of production, but it is usually achieved by increasing the number of platelets in the separated plasma [27].

Various methods are used to measure the platelet concentration in PRP. One of the most common methods is an automated blood analyzer that can accurately measure the platelet count in plasma. Other methods include using microscopy to count platelets directly and using special cats for immunological detection of platelets using antibodies against specific platelet markers [28].

PRP contains not only platelets but also other biological components that play an im-

portant role in the process of tissue regeneration. These components include growth factors, cytokines, anti-inflammatory molecules, and extracellular matrices [29].

The growth factors (FGF, EGF, PLGF and others) are present in PRP and promote cell proliferation, differentiation, and synthesis of extracellular matrices. These growth factors can activate various cellular pathways and promote tissue regeneration. In addition, PRP contains other biological components that may have anti-inflammatory effects, such as interleukins and TGF- β . These molecules help to control the inflammatory response and promote tissue healing [30].

A team of scientists led by P. R. Amable tested various centrifugal rotation conditions (RCF), including rotation speeds ranging from 240 to 400 \times g, duration from 8 to 19 minutes, and temperatures from 80 to 160 °C. A centrifuge with a cooling system was used in all stages of the experiments. The optimal results were achieved when using the parameters 300 \times g for 5 minutes at 120 °C and 240 \times g for 8 minutes at 160 °C for the first centrifugation step. For the second centrifugation, a speed of 700 \times g for 17 minutes was used to reduce platelet loss. The removal of 2/3 of the residual plasma during the second centrifugation resulted in a significant increase in platelet count (70-80 %) and a fivefold increase in the platelet concentration. In this study, the researchers managed to restore 46.9 to 69.5 % of the total number of initial platelets, and the procedure led to a 5.4-7.3-fold increase in platelet concentration (from 1.4×10^6 to 1.9×10^6 platelets/ μ L) [47].

A study by Amanda *et al.* [48] highlights the key considerations during the centrifuga-

tion step to achieve consistent and high-quality results. The blood samples were obtained from 20 healthy donors who provided informed consent. Two centrifugation steps were investigated, focusing on the impact of centrifugal acceleration, duration, processed volume, and platelet gradient. PRP was assessed based on platelet concentration, integrity, and viability (measured by sP-selectin). Lower centrifugal accelerations were found to be advantageous for optimal platelet separation. Specifically, processing 3.5 mL of blood at 100 \times g for 10 minutes (1st spin), followed by 400 \times g for 10 minutes (2nd spin) and withdrawing 2/3 of the remaining plasma, resulted in a notable increase in platelet recovery (70–80 %) and concentration (5x), while preserving the platelet integrity and viability. However, a decrease in the platelet recovery was observed when processing a larger whole blood volume (8.5 mL). The study underscores the importance of factors such as centrifugal acceleration, processing time, volume of whole blood, and minimizing the platelet gradient before sampling to ensure consistent compositions within the autologous nature of PRP.

Mazzocca *et al.* [49] discuss a study aimed at quantifying the levels of various blood components in the platelet-rich plasma (PRP) products, in particular, comparing a one-stage (using clinically used commercial devices) and two-stage separation systems. The authors also examined the impact of three consecutive blood draws on the final PRP composition. They used three different PRP separation methods, including two single-spin processes (PRP_{LP} and PRP_{HP}) and a two-spin process (PRP_{DS}), using blood samples from eight subjects with a mean age of 31.6 ± 10.9 years. The

platelet, red and white blood cell concentrations, as well as growth factors, were evaluated for each separation method. Additionally, the work studied how three repeated blood draws affect the components of PRP. The results showed significant differences in the content and concentration of platelets, leukocytes and growth factors between the different separation methods. Concerning the total platelet count, all separation systems exhibited a significant increase in the number of platelets compared with the native blood ($142.7 \pm 44.40 \times 10^3/\text{mL}$). Particularly, PRP_{HP} ($873.8 \pm 207.82 \times 10^3/\text{mL}$) demonstrated a significantly higher platelet count compared to both PRP_{LP} ($378.3 \pm 58.64 \times 10^3/\text{mL}$) and PRP_{DS} ($447.7 \pm 183.7 \times 10^3/\text{mL}$) ($p \leq 0.05$). No significant difference in platelet count was observed between PRP_{LP} and PRP_{DS} ($p = 0.52$). Regarding the red blood cell concentration, native blood exhibited the highest level ($4.1 \pm 0.4 \times 10^6/\text{mL}$), which differed significantly from all other separation methods. PRP_{LP} ($0.2 \pm 0.1 \times 10^6/\text{mL}$) and PRP_{DS} ($0.02 \pm 0.04 \times 10^6/\text{mL}$) were not significantly different from each other. However, both PRP_{LP} and PRP_{DS} groups had significantly fewer red blood cells compared to the PRP_{HP} group ($1.0 \pm 1.4 \times 10^6/\text{mL}$). All separation methods resulted in a significant increase of the platelet concentration compared to native blood. The PRP_{HP} procedure demonstrated significantly higher platelet and leukocyte concentrations compared to the one-step PRP_{LP} procedure and the two-step PRP_{DS} procedure. Although no significant differences were observed between PRP_{LP} and PRP_{DS}, wide intra-individual variations in platelet and white blood cell counts were noted when the results of the three blood draws were evalu-

ated. The study suggests that single-stage procedures are capable of generating sufficient platelet counts for clinical use. However, regardless of the separation method, the variations in platelet counts, leukocyte counts and growth factor levels were observed. The component variability and its potential impact on dosing should be considered for both single and sequential administrations of platelet-rich plasma. The observed differences in components between different separation methods may have a specific effect on the tissue being treated.

The main goal of the study by Chernyshenko *et al.* [50] was to develop a simple and effective method for producing highly concentrated platelet-rich plasma (PRP) with a concentration exceeding $1 \cdot 10^6$ 1/mL, suitable for biomedical use. By optimizing the amount of heparin as an anticoagulant, the researchers achieved a final concentration without compromising the platelet reactivity. The resulting PRP, concentrated fivefold with a total cell concentration of $1 \cdot 10^6$ 1/mL, showed a significant rate of aggregation upon stimulation, indicating that the platelets retained the ability to release the biologically active compounds during cell therapy. It was also found that lower concentrations of heparin minimized the risk of injection site haemorrhage, confirming the biomedical suitability of the developed PRP product.

Shevchenko and Rublenko examined the autologous regenerative products, in particular platelet-rich plasma (PRP) and platelet-rich fibrin (PRF), in the context of regenerative medicine [51]. The study was focused on the effect of different centrifugation parameters on the concentration and histologi-

cal characteristics of the platelets in fibrin clots. Blood was drawn from rabbits, and PRP and PRF were obtained at different values of the centrifugal force. The study showed that the optimal centrifugal force for achieving the highest platelet concentration in PRF is 100 g, which exceeds $800 \times 10^9/\text{mL}$. It was found that changing the centrifugation parameters affects the length and concentration of platelets in fibrin clots, which indicates the importance of centrifugation conditions for obtaining effective regenerative biomaterials.

To summarize, PRP contains not only platelets but also various biological components such as growth factors, cytokines, and anti-inflammatory molecules playing an important role in the tissue regeneration. These components work synergistically to improve the regeneration and repair of muscle tissue.

Mechanisms of PRP effect on muscle regeneration

One of the mechanisms by which PRP promotes muscle regeneration is the anti-inflammatory effect. After a muscle injury, the inflammatory process occurs, which can slow down tissue healing. PRP contains anti-inflammatory molecules, such as interleukins and TGF- β , which help to control the inflammatory response [3, 4].

These anti-inflammatory molecules interact with the cells in the immune system, reduce the production of inflammatory mediators, and help to reduce inflammation. This allows the creation of a more favorable environment for the muscle tissue regeneration and reduction of the damage caused by the inflammatory process [4].

PRP contains growth factors that can stimulate the cellular proliferation and differentiation of affected muscle tissue. These growth factors activate the signaling pathways that control the cellular activity and promote the muscle cell division to repair the damaged tissue [18].

In addition, PRP can attract and activate fibroblasts, which play an important role in the production of extracellular matrices. This helps to restore the muscle tissue structure and develop new muscle fibers [31].

PRP also affects the synthesis of collagen, the main component of the extracellular matrix of muscle tissue. The growth factors present in PRP activate fibroblasts to produce collagen, which promotes tissue healing and restores the muscle fiber structure [32].

PRP promotes the development of new blood vessels in the damaged muscle tissue, which is called angiogenesis. The growth factors present in PRP stimulate the formation of new blood vessels, which improves the supply of blood, oxygen and nutrients to the affected muscle tissue and promotes recovery.

Clinical studies on the effect of PRP on muscle regeneration

In a clinical trial involving humans, scientists investigated the impact of Platelet-Rich Plasma (PRP) on the recovery of strained muscles. The results revealed an expedited healing process and improved muscle functionality following the application of PRP. PRP influences the healing process by stimulating early vascularization, leading to increased rates of ligamentization. PRP is rich in growth factors that facilitates the tissue repair and is extensively used in the fields of orthopedics and sports

medicine. Several studies have indicated that PRP promotes muscle recuperation, reduces pain and swelling, and shortens the time required to return to sports activities [14, 22, 27, 33, 34].

Another significant study was conducted by Gessica Giusto *et al.* In this study, eight female pigs underwent jejunum-jejunal anastomoses, with two as controls and four treated with either PRP or PRGF at higher platelet concentrations. After 8 days, evaluations were conducted on adhesions, leakage, bursting pressure, and histological appearance. The results showed no significant difference between PRP and PRGF treatments, except for improved mucosal epithelization in the PRGF group. Overall, both PRP and PRGF are safe and cost-effective options but did not demonstrate a clear advantage over no treatment, except for mucosal epithelization in the PRGF group [35].

Several clinical trial results have confirmed the effectiveness of PRP in regenerating muscle tissue. The studies generally showed that the patients who received PRP therapy recovered faster and had better muscle function compared to the patients who did not receive this therapy [3, 9, 11, 36]. The study also confirmed that PRP promoted healing and repair of muscle tissue in animals [4, 18, 38, 39].

MSCs are a new modern and potentially promising material for treating the muscle injuries [40]. MSCs, like PRP, are being studied for their potential use in the regenerative medicine. MSCs have unique characteristics, including the ability to self-renew and differentiate into different cell types. They have shown promising results in the clinical trials for neurodegenerative and autoimmune diseases. While both MSCs and PRP have po-

tential therapeutic applications, there are the challenges that need to be addressed, such as cell types, administration and production methods [41, 42]. Further research is needed to fully understand and optimise the use of MSCs and PRP, or their possible combination in the regenerative medicine.

Prospects of using PRP in clinical practice

The key aspects of using PRP in clinical practice are the optimal dosage and timing of PRP administration to achieve the best results in the muscle tissue regeneration [3]. Several researchers are actively studying the impact of factors such as the platelet concentration in PRP, the amount of other biological components of PRP, centrifugation modes, and retention time on the treatment effectiveness [43, 44]. In addition, the study of the optimal centrifugation time and PRP activators is also an important factor that can affect the regenerative properties of PRP.

The use of PRP in regenerative medicine has both advantages and limitations. The advantages are its naturalness, high biocompatibility, low toxicity, and minimal side effects. PRP can also be beneficial from an economic point of view, as it can be obtained from the patient's own blood. In addition, PRP can be used in combination with other treatments to improve their effectiveness [22, 45].

However, the use of PRP also has some limitations. For example, the lack of standardized protocols and variations in the protocols for obtaining PRP may affect its effectiveness. Also, more research is needed to establish the optimal dosages and regimens of PRP administration for different types of muscle injuries.

In addition, the availability of PRP production technologies may be limited in certain clinical settings.

Future research should focus on improving PRP treatment protocols for the muscle tissue regeneration. It is important to establish the optimal dosage, time of administration, and time intervals for repeated PRP administration for different types of muscle injuries. Additional research should also be conducted to establish the mechanisms of PRP action at the cellular level and molecular processes that occur during the muscle tissue regeneration.

Besides, it is important to compare the effectiveness of PRP with other methods of treating the muscle and sports injuries, such as usage of stem cells and other biological materials. This will help to establish optimal treatment approaches and to contribute to the further development of the regenerative medicine.

Conclusions

Considering the studies discussed above, it may be concluded that PRP has significant potential to stimulate the muscle tissue regeneration. Its use contributes to the anti-inflammatory effects, increased the cell proliferation and differentiation, as well as enhanced the collagen production and the development of new blood vessels. These mechanisms promote the healing and repair of damaged muscle tissue.

PRP is proved to be a promising tool in clinical practice to improve the muscle tissue healing. It can be effective in the treatment of sports injuries, muscle trauma, and other musculoskeletal disorders. The PRP usage is a safe, natural, and minimally invasive method because of using the patient's own blood. The

prospects for the PRP use include the development of standardized treatment protocols, the optimization of dosage and timing of administration, and the expansion of research to determine its effectiveness in various clinical scenarios.

In general, the use of PRP in clinical practice may open up new opportunities for the muscle tissue regeneration and improve outcomes of the muscle injuries. The further research and the development of treatment protocols will help to clarify the optimal parameters of the PRP use and to expand its application in clinical practice.

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Можливості та вплив збагаченої тромбоцитами плазми (PRP) на регенеративні властивості м'язової тканини

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Збагачена тромбоцитами плазма (PRP) часто використовується в різних галузях медицини. Сучасний стан

використання даного методу має великі перспективи для розвитку. PRP може покращити перебіг багатьох захворювань. У цій статті мова піде про вплив PRP на регенеративні властивості м'язів. Існує багато протоколів для підготовки PRP, кожен з яких має свої стандартизовані параметри і заявлені результати. У статті представлено огляд літератури щодо застосування плазми, збагаченої тромбоцитами, фокусується увага на визначенні PRP, різних методах підготовки та активації, а також концентрації факторів росту.

Ключові слова: збагачена тромбоцитами плазма, регенерація м'язів, відновлення, травми м'язів, репарація тканин.

Received 15.09.2023