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## Summary

The physiological support of blood-derived products for tissue repair and regeneration is gaining attention for many robust applications in the field of regenerative medicine. Platelet-rich plasma (PRP) is one of the prominent blood-derived product widely used for in vitro cell culture supplementation and therapeutical interventions. The amount of growth factors and cytokines present in the platelet alpha granules from PRP provides all necessary anabolic factors to maintain cell propagation, cell differentiation, and phenotype stabilization for in vitro cell culture. Divergences existing from different preparation protocols of PRP is consequential to existing various sub-categorical plasma enriched products across laboratories worldwide. This review outlines the background, methodologies, and current knowledge about human blood-derived products and underlines its advantage and disadvantage in a wide range of applications.

**Keywords**
Blood derived products– Platelet rich plasma– Osteoarthritis

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## Blutderivate – Hintergrund, Technologie und Anwendung in Osteoarthrose

## Zusammenfassung

Die physiologische Unterstützung Blut abstammender Produkte für die Gewebereparatur und -regeneration gewinnt bei vielen Anwendungen im Bereich der regenerativen Medizin an Aufmerksamkeit. Thrombozytenreiches Plasma (PRP) ist eines der bekanntesten Blutprodukte, das häufig als Supplement in der In-vitro-Zellkultur und für therapeutische Anwendungen verwendet wird. Die Menge an Wachstumsfaktoren und Zytokinen, die im Thrombozyten-Alphagranulat von PRP vorhanden ist, liefert alle notwendigen anabolen Faktoren, um die Vermehrung, Differenzierung

REVIEW / SPECIAL ISSUE

# Blood derived products – background and technology and clinical application in osteoarthritis

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## Introduction

The origin of blood-derived products dates back to the invention of biological fibrin glues and fibrin sealants for therapeutic applications. Their use as a hemostatic agent was reported in 1909[1] and later as a fibrin suture for peripheral nerve regeneration in 1940.[2] Then it was determined that its regenerative effect was due to the polymerized fibrin gel, and not on the presence of platelets in plasma solutions. Although the term "platelet-rich plasma" was already used by Dr. Kingsley in 1954 (to designate thrombocyte concentrate during experiments related to blood coagulation[3]), [4], blood products with a high platelet concentration and thus, enhanced growth factor levels were gaining interest in the field of regenerative medicine since 1998. Robert E. Marx[5,6] published the first clinical report of PRP used for the improvement of bone formation. Platelet-rich fibrin (PRF) is a product based on the strong fibrin matrix[7] that gave to the rise of various new blood-derived products with modified composition and preparation methods. During this time, perplexity on PRP nomenclature started. The classification about platelet concentrates has been proposed yet there are many questions left unanswered, and the debate on blood product still endures.[8] In sportsmedicine the hype about PRP began in 2009 after the Super Bowl, where Hines Ward, a football player from the Pittsburgh Steelers won the game after a PRP treatment reported in New York Times. However, there are still many questions left unanswered and the blood product debate continues.

## Four principal families of blood product derived biologics

Autologous blood-derived products often termed under their acronyms PRP (Platelet-Rich Plasma) or PRF (Platelet-Rich Fibrin), have become a standard treatment procedure. The products are blood extracts obtained from the processing of blood samples, mostly through centrifugation. The process aims to separate the blood components in order to discard elements considered as not suitable (erythrocytes) and to

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und den Phänotyp von Zellen *in vitro* aufrechtzuerhalten. Abweichungen, die aufgrund verschiedener Herstellungsprotokolle von PRP entstehen, resultieren in einer großen Anzahl verschiedener Plättchen angereicherten Plasmaprodukte in Laboratorien weltweit. Dieser Review gibt einen Überblick über den Hintergrund, die Methoden und das aktuelle Wissen über menschliche Blutprodukte und unterstreicht deren Vor- und Nachteile in einer Vielzahl von Anwendungen.

**Schlüsselwörter**
Blutprodukte – Plättchenreiches Plasma – Arthrose

concentrate the elements that may be used for therapeutic applications (fibrin, platelets, growth factors or leukocytes) (fig. 1). All these blood derivatives, regardless of their preparation methodology, are prepared from the whole venous blood and are applied to a lesion or accelerate wound healing. The official classification of blood-derived products, proposed in, is now widely quoted to harmonize the terminology. This classification defines four leading families of blood products.

- **Platelet-Rich Plasma (PRP)** – platelet-based blood derivative, poor in leukocytes, and having
- a low-density fibrin network, after activation.
- **Leukocyte containing Platelet-Rich Plasma (L-PRP)** platelet based blood derivative
- containing leukocytes, and having a low-density fibrin network, after activation.[2]
- **Platelet-Rich Fibrin (PRF) – or Leukocyte-Poor Platelet-Rich Fibrin** – products poor in
- leukocytes and having a high-density fibrin network.
- **Leukocyte- and Platelet-Rich Fibrin (L-PRF)** products containing leukocytes and having a high-density fibrin network.[2]

## Methods of preparation and delivery of PRP

The variation in preparation of PRP is quite considerable due to different protocols across laboratories and clinics. However, most of the preparations include by withdrawing autologous whole blood with anticoagulant citrate before centrifuging the whole blood. Commonly used anticoagulants include citrate or dextrose and these anticoagulants facilitate the separation of platelets without disrupting their granular molecules. Several systems do not contain an anticoagulant, but the cell-free plasma that contains the clotting factors and without the addition of anticoagulants could result in clotting of the PRP when further administered into the site. PRP with or without an activator has commonly been used, and likewise, leukocyte-rich or leukocyte-poor PRP have been used.

PRP products can be used as liquid formulations or as an activated gel form, thus allowing them to be injected at the site of the wound or delivery of the activated gel form.[9] In general, the PRP preparation protocol requires the collection of venous blood with an anticoagulant, followed by two-step centrifugation. After the first centrifugation, PPP (platelet-poor plasma) is collected, and the bottom layer with erythrocytes is discarded, then the second centrifugation is used for platelet concentration. By definition, PRP is a platelet concentrate that typically contains a 3-5-fold increase of platelets compared to the whole blood. PRF is a fibrin matrix in which activated platelets are trapped, together with growth factors and cytokines, which are gradually released to the serum fraction. This product only exists in a solid gel form and therefore, cannot be introduced to the injury site by injection. However, the stable fibrin clot allows for the use of this product as a solid material in other applications. The protocol for PRF preparation is one-step centrifugation in a glass tube without any anticoagulants. It is essential to centrifuge the sample immediately after blood collection for obtaining a clinically usable PRF. Blood starts to coagulate upon contact with the glass. Therefore, centrifugation is required to concentrate fibrinogen in the middle and upper part of the tube before the circulating

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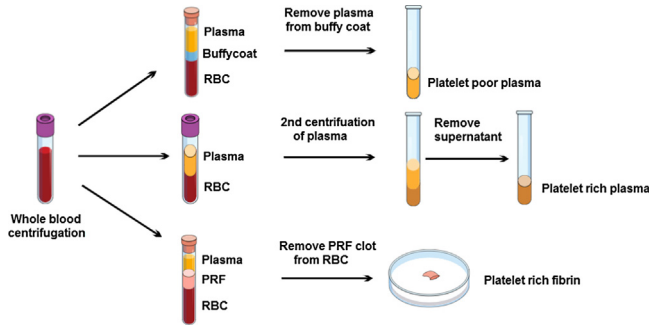


Figure 1
**Schematic representation of preparation of buffy coat -based platelet poor plasma (PPP), double centrifugation-based platelet rich plasma (PRP) and activation-based platelet rich fibrin (PRF).**

thrombin transforms it into fibrin. Otherwise, the fibrin polymerizes diffusely, and only a small blood clot without a structured fibrin network is obtained. After driving out the serum from fibrin matrix, the PRF membrane is ready to use as a membrane or in conjunction with bone grafts; promoting wound closure and healing, growth, and maturation of bone, and haemostasis. [10]

## Cell-free blood products

In this approach, blood coagulation is induced, and the final product is a serum containing high concentration of growth factors. The use of cell-free blood product decrease or eliminate the potential risks that come with the use of very concentrated platelet-rich plasma derivatives.

## Autologous conditioned serum (ACS)

ACS has been developed based on the idea given by Meijer et al., for stimulating the production of anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1Ra) by blood cells.[3] IL-1-Ra is an important mediator of inflammation and a limiting factor for tissue destruction in various musculoskeletal conditions.[11] High concentration of IL1Ra was observed in cell-free serum supernatant; obtained after centrifugation of the coagulated blood, after a 24 hour incubation period, with the glass beads set at 37 °C. ACS has shown efficacy in the treatment of knee and hip osteoarthritis (OA), in randomized controlled trials.[4] However, due to the thermal manipulation of blood during preparation, this procedure is not permitted in the United States.

## Hyperacute Serum

Hyperacute serum (HAS or hypACT), is a serum-based blood product free of platelets, leukocytes, and fibrin. HAS is obtained through coagulation induced immediately after blood collection in a single step centrifugation process and squeezed out from the fresh fibrin clot. Growth factors released during the platelet activation remain in the serum; however, the overall concentration of cytokines and growth factors is lower than in PRP. It was observed that hyperacute serum stimulates cell proliferation, supports adipogenic differentiation, preserves the viability of bone, synovium, and cartilage and decreases the pro-inflammatory cytokine levels *in vitro*. [12,13]

## Biologically active molecules in blood derived products

Platelets contain various growth factors in their α-granules, such as PDGF, EGF, TGF-β1, IGF-1, VEGF, bFGF and HGF, which are known to be involved in healing and regeneration processes.[14,15]Therefore, growth factor-rich blood products can significantly improve tissue healing. PRP is a product that contains concentrated platelets in a suspension of plasma. PRP is very often obtained from an individual's whole blood that is further centrifuged to separate the whole blood towards its constituents mainly the plasma, platelets, leukocytes, red blood cells. The principle idea behind the usage of PRP is that the highly concentrated platelets will be ruptured hence releasing a pool of growth factor, cytokines which in turn could facilitate the healing depending on the clinical intervention.

The most common growth factors released by PRP include transforming growth factor-β (TGFβ), Insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF-BB), vascular endothelial growth factor (VEGF) .[16] Chondrocytes when cultured *in vitro* supplementation with PRP secreted more PDGF -BB and TGF-β1 in a correlation to reduction in IL-1βlevels as well a stable release of BMP-2.[17,18]

Platelet concentration in PRP indeed differs from donor to donor compared their baseline in the whole blood. Overall when platelets are concentrated in PRP by centrifugation, they are 6-8 times concentrated than the baseline found in

**Table 1.** Growth factors in PRP and their functions.

| Growth factor | Function |
|---|---|
| PDGF (platelet-derived growth factor) | Fibroblast and smooth muscle cell mitogen[21] Involved in wound healing[21] Regulates the secretion and synthesis of collagen[22] |
| EGF (epidermal growth factor) | Fibroblast, endothelial cell and keratinocyte mitogen Involved in the healing of chronic wounds[21] |
| TGF-β1 (transforming growth factor-β1) | Stem cell, fibroblast, smooth muscle cell and osteoblast mitogen[21,22] Promotes angiogenesis and extracellular matrix production[21] |
| IGF-1 (insulin-like growth factor-1) | Regulates bone maintenance Important modulator of cell apoptosis[21] |
| VEGF (vascular endothelial growth factor) | Promotes angiogenesis Promotes healing of chronic wounds Supports endochondral ossification[21] |
| bFGF (basic fibroblast growth factor) | Angiogenic factor[21] Stem cell, chondrocyte, osteoblast and endothelial cell mitogen[22] |
| HGF (hepatocyte growth factor) | Anti-inflammatory effect on injured organs[23] |

whole blood. However, there is no study indicating a positive correlation between elevated growth factor levels to highly concentrated platelets. Most of the studies have understood that different ways of rupturing the α granules from the platelet do have a difference of elevated of less growth factor levels (mechanical disruption by sonication, repeated freeze-thawing). BMP-2 is an excellent choice of biologic than TGF-β1 to avoid dedifferentiation in chondrocytes and hypoxia favors BMP-2 mediated type II collagen expression.[19] Similarly cold pre-conditioning of PRP at 4○C preferably enhances the angiogenic factors such as VEGF, IGF-1 than the traditional method of freeze thawing at 37○C to produce PRP releasates.[20]

Activation of platelets is often a misinterpretation that is compared to rupturing of platelets.Platelets get activated during the clotting process of the blood and start to secrete the growth factors usually within 10 minutes if the clotting process. About 95% of those factors are released post 1 hour of activation. Some of the PRP preparations use thrombin to activate platelets to form a clot and release the solution that contains all the growth factors. Others use anticoagulants like heparin, sodium citrate, citrate dextrose to prevent clotting and obtain the growth factor rich PRP solution.Regarding PRP preparation methods, there is a huge debate on agreeing to a common consensus

that standardizes the centrifugation speed, force, single spin or second spin and activation, rupturing methods to understand the clinical efficacy when PRP is intervened clearly. Some of their most important functions, affecting the regeneration process, are shown in Table 1.

## Limitations of blood products

PRP and PRF are used in clinics as autologous products; therefore, they are relatively low-risk treatments, with the potential to improve and speed healing processes.[24] However, Magalon et al., demonstrated substantial differences among platelet-rich plasma products produced by various automated and manual protocols described in the literature.[25] These observations raise a concern, that various PRP products may evoke diverse cellular reactions due to the varied content of platelets, leukocytes, and erythrocytes. In many preclinical or even clinical studies, the actual concentration of injected PRP is not always specified, which makes the preparation of a universal and effective protocol very difficult. Platelet number correlates with growth factor dose, which in turn determines the actual biological response. Graziani et al., already observed inhibition of cell proliferation using a high platelet concentration. Studies of Broderick et al. and Harten et al., showed that with a high level of TGF-β1 or VEGF, bone formation was inhibited.[26] There are also some concerns about the leukocytes content in PRP. Some

studies reported that leukocytes improve the healing process, whilst others showed the correlation between leukocyte content and high levels of pro-inflammatory cytokines.[27] Xiong et. al., compared leukocyte poor PRP formulations from 39 young male and female healthy patients for difference cytokine levels for gender and age differences which revealed that inflammatory cytokines such as IL-1 β, TNF-α, IL-1Ra were higher in male candidates than female candidates.[28] In the same study IGF-1 was differently higher in younger patients than older patients indicating that intra-individual differences and age compromise the level of constituents in PRP despite the similar preparation method. In another study it was indicative that a 20 minute vigorous exercise program significantly increased the platelet concentration in buffy coat derived PRP along with higher hematopoietic stem cells as well as plasma based PRP which shows a potential to increase the yield of PRP.[29]In the case of PRF, if intended for injection, the solid-fibrin matrix has to be fragmented at first. Therefore, the use of cell-free autologous blood products can serve as an alternative to PRP and PRF treatment. Before any blood product can be used in medical treatment, it should be tested using *in vitro* biological systems, like human cells or explant cultures. There are some limitations to PRP and PRF when using them as replacement for a standard fetal calf/bovine serum supplement. In common monolayer cell culture systems, PRP has to always be used with anticoagulants; otherwise, the cell culture medium becomes a gel. PRF is a solid-fibrin membrane, so it is difficult to use it as a traditional supplement in monolayer cell culture systems. Serum-based blood products are cell-free, do not need any anticoagulant and remain in liquid form; therefore, their use is more convenient for *in vitro* systems.

## PRP for Intra-articular applications in Osteoarthritis

In an FDA-approved, randomized, double-blind, placebo-controlled clinical trial led by Smith et al. , [30] ACP was intra-articularly injected in 30 patients (15 patients ACP, 15 patients saline placebo) with a level of incidence 1 treated with 3 weekly injections and followed for 12 months based on the WOMAC score. Outcomes revealed that ACP groups improved the overall WOMAC scores by 78% from their baseline score compared saline placebo group which improved only by 7% after 1 year and further no adverse events for ACP treatment were reported.

Based on in vitro trials reported so far, clinicians argue that leukocyte-poor PRP has to be taken into consideration. However, the only clinical trial reported so far could not elucidate the differences between the two preparations on their superiority. Filardo et al. [31] compared platelet rich growth factor (PRGF) versus PRP in 144 symptomatic patients affected by degenerative cartilage lesions and OA in a quasi-experimental study. 72 were treated with 3 weekly injections of PRGF (platelet concentrate) prepared by a single spinning procedure, the other 72 with 3 weekly injections of PRP prepared by a double-spinning approach. Patients were evaluated at 2, 6, 12 months based on IKDC, EQ-VAS, and Tegner scores. No differences were found between treatment with PRGF or PRP. The satisfaction level was similar too: 76.4% in PRGF group and 80.6% in PRP group.

In a multicenter, double-blinded clinical trial Sachez et al. [32] compared PRGF Endoret versus HA in randomly assigned 176 patients (3 injections weekly). Secondary outcomes were measured based on the WOMAC Index and the OMERACT-OARSI and evaluated up to 12 months (time points; 1, 2, 6 months). Patients receiving PRGF-Endoret showed 14.1% points significantly higher than patients receiving HA.

In a randomized controlled trial, with a level of incidence 1, Cerza et al. [33] compared autologous conditioned plasma (ACP) versus PRP in 120 patients (60 and 60) who received 4 intra-articular injections once a week for 4 weeks. The outcomes were measured based on WOMAC scores before infiltration and at 1, 3, up to 6 months after the first injection. Outcomes measured from the WOMAC revealed that treatment with ACP significantly exhibited a better clinical outcome than treatment with HA.

Filardo et al. [34] compared the effect of PRP versus HA for viscosupplementation in a degenerative joint disease. 109 patients (55 patients with HA and 55 patients with PRP) were treated and evaluated up to 12 months. No significant differences occurred between both the groups except in a few patients with less degenerated joints (based on kellgrence score of 2.0) had a tendency of better results with PRP however without reaching statistical significance indicating that patients under 50 years have a greater chance to benefit from PRP based growth factor supplementation.

In another clinical trial, Filardo et al. [35] performed a study design with a randomized controlled trial with a level evidence of 1 comparing

PRP versus HA. Here 192 patients were treated (96 with PRP and 96 with HA) with a follow-up of 12 months. The study was powered to detect a minimum clinically significant difference of 6.7 points on the IKDC score. Both PRP and HA groups demonstrated improvement regarding IKDC score and did not differ at any follow-up time points (2, 6, 12 months). PRP was noted with more swelling and pain post injection for the first few days, but the effects lasted only for the few days. The PRP and HA group did not differ regarding any secondary outcome as observed in their earlier study.
Recent studies comparing comparing PRP versus HA in a double blind randomized setting[36] showed better outcomes clinically and in intra-articular biology in knee osteoarthritis and that the actual status of the cartilage damage is not predicable for effectiveness of PRP treatment [37].

## Conclusion

Blood derived products opens many potential applications in the field of regenerative medicine with supporting evidence from several studies for their capacity to regenerate several cell types in vitro. The intra-articular injection of PRP is a viable treatment for knee OA and has the potential to lead to symptomatic relief for up to 12 months and beyond. There appears to be an increased risk of local adverse reactions after multiple PRP injections but diminishes eventually. PRP offers symptomatic relief to patients with early knee degenerative changes, and its use should be considered in patients with knee OA, maybe not for advanced stage OA. Future considerations should focus on a pure PRP alleviating the leukocyte content in them. More controlled trials and experimental work are for sure necessary to clarify clear indications for PRP use and even more to standardize the technology of PRP-production to allow a more defined composition of blood derived products.

## Conflict of interests

There is no conflict of interest.

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