The use of autologous platelet-leukocyte gels in enhancing the healing process in surgery, a review.

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ABSTRACT

Background:
The therapeutic use of autologous prepared platelet-leukocyte enriched gel (PLG) is a relatively new technology in the stimulation and acceleration of soft-tissue and bone healing. The effectiveness of this procedure lies in the delivery of a wide range of platelet growth factors, mimicking the physiological wound healing and reparative tissue processes. Despite an increase in PLG applications, the structures and kinetics of this autogenous derived biological material have not been observed.

Materials and Methods:
A review of the most recent literature was executed in order to evaluate the use of PLG in various surgical disciplines.

Results:
The review revealed that the application of PLG has been extended to various surgical disciplines, including orthopedics, cardiac surgery, plastic, and maxillofacial surgery, and recently also to endoscopic surgery.

Conclusion:
This review demonstrates the usefulness of PLG in a wide range of clinical applications to improve healing following surgical procedures.
INTRODUCTION

Soft tissue wound healing and bone growth involves physiological cascades in which cellular and hormonal factors play pivotal roles\textsuperscript{1,2}. Some of these cascade components can be isolated from autologous drawn whole blood. Point-of-care devices can intra-operatively fractionate the autologous blood into platelet-poor plasma, platelet-leukocyte-rich plasma (P-LRP), and red blood cells\textsuperscript{3,4}. The P-LRP fraction is a mixture of concentrated platelets and leukocytes. P-LRP can be activated by (autologous) thrombin to create a viscous solution termed platelet-leukocyte gel (PLG). This platelet coagulum can be exogenously applied to soft wound tissues, bone, or synthetic bone as a spray or as a solid, clotted, gelatinous mass. The rationale for applying platelet gel is based on the delivery of platelet growth factors to tissues, and on the fact that platelet alpha granules ($\alpha$-granules), which are found inside the platelets, contain a variety of growth factors\textsuperscript{5}. Platelet gel growth factors are peptides that promote cell proliferation, differentiation, chemotaxis, and the migration of various cells involved in both wound healing and bone growth\textsuperscript{6,7}.

Recently, numerous P-LRP devices have become available for therapeutic use to stimulate and accelerate soft-tissue and bone healing and to control postoperative wound bleeding. The rationale for applying PLG lies in mimicking and accelerating physiological wound healing and reparative tissue processes.

In this article we provide information on the results of electron microscopic imaging used to evaluate the content of PLG. Furthermore, since the use of PLG is a relatively new peri and/or peri-operative biotechnological application, new and more indications to use PLG will be defined. Therefore, new PLG applications are reviewed for different applications, including cardiac surgery, general surgery, orthopedics and traumatology, cosmetic surgery, maxillofacial surgery, sports medicine, and endoscopic surgical procedures.

DEFINING PLATELET-LEUKOCYTE RICH GEL.

Platelet-leukocyte gel is prepared from a high concentration of platelets which have been acquired from freshly drawn autologous whole blood. In general, this blood is obtained by point-of-care devices and is sequestered into different blood components being platelet-leukocyte-rich plasma (P-LRP), platelet poor plasma, and erythrocyte concentrate\textsuperscript{8}. One of the characteristics of P-LRP is that it consists of a small volume of plasma with fibrinogen, platelets and leukocytes. Platelets suspended in the P-LRP are in an inactive state and in liquid form. The platelet growth factors are found inside $\alpha$-granules, which are present in the platelet cytoplasm. Platelets become immediately activated because of interaction
with thrombin, the most potent platelet activator, where after a sticky platelet aggregate is formed. Subsequently, platelet $\alpha$-granules release growth factors into the extra cellular environment where they bind to specific platelet growth factor receptors. Following this, through intracellular tissue signaling, a number of pathways are triggered that initiate the healing process.

Apart from the presence of a high concentration of platelets in the PLG, several differentiated and non-activated leukocytes are present at high levels. In particular this includes neutrophilic granulocytes and monocytes, both known for host-defense mechanism actions against bacteria, through the action of myeloperoxidase that creates hypochloric acid. The platelet numbers in the PLG are 3-7 times higher than normally found, while the white blood cell count is 2-4 times greater than normal$^{9,10,11}$.

**Healing Mechanisms of Platelet-leukocyte Gels.**

It is generally accepted that platelet growth factors play a central role in the healing process and in tissue formation$^{12}$. Wound healing is a well orchestrated and complex series of events involving cell-cell and cell-matrix interactions, where platelet growth factors serve as messengers to regulate various processes. Initially tissue repair begins with platelet clot formation, activation of the coagulation cascade and platelet degranulation and the release of growth factors. Following release, specific platelet growth factors will bind to particular target tyrosine growth factor receptors, which subsequently activate intracellular signal transduction pathways$^{7,13}$. During the first two days of wound healing an inflammatory process is initiated by migration of neutrophils and subsequently macrophages to the wound site. In turn, activated macrophages release multiple growth factors, including platelet derived growth factor (PDGF), transforming growth factors-alpha and -bèta (TGF-\(\alpha\), TGF-\(\beta\)), interleukin-1 (IL-1), and fibroblast growth factor (FGF)$^{11}$. Angiogenesis and fibroplasia starts shortly after day three, followed by collagen synthesis on day’s three to five. This process leads to an early increase in wound breaking strength, which is the most important wound healing parameter of surgical wounds, followed by epithelialization and the ultimate remodeling process$^{14}$.

Some of the functions of platelet growth factors in tissue repair revealed that a controlled sequential appearance is crucial following primary wound closures after surgical procedures, for the treatment of bone and cartilage defects, muscle and tendon lesions and to promote synthetic tissue ingrowth during reconstructive surgeries$^{15}$.
Based on the actions of the various platelet growth factors during the different stages in the wound healing cascade, the use of autologous PLG to stimulate wound repair is an interesting proposition, although recombinant growth factors have been used to stimulate wound healing. However, when compared to recombinant single growth factor applications, platelet gels have the supreme advantage that they synergistically induce various growth factors and promote mitogenesis of mesenchymal stem cells at the wound site\textsuperscript{16,17}.

**NOVEL PLATELET-LEUKOCYTE GEL APPLICATIONS**

Following the activation of P-LRP with thrombin, a viscous gel with a degree of plasticity, is formed that will stick to wound tissues. At this stage the PLG is exogenously applied, by using a syringe delivery technique, to surgical wound sites during closure, to soft tissue structures to stimulate tissue regeneration (Figure 1A-B), or it is mixed with bone or bone substitutes to accelerate bone healing.

![Figure 1](image)

*Figure 1. Using a double syringe delivery technique, platelet-leukocyte gel is applied on a skin wound in a patient with decubitis ulceration.*

Outcome of multiple studies have been published on the efficacy of PLG treatment\textsuperscript{18-20}. Proponents of the application of PLG refer to improved wound healing and increased bone growth to warrant its use. A reduction in the development of severe postoperative wound infections has been reported when PLG was applied during incisional wound closure following a cardiac surgical procedure\textsuperscript{21}. As a result, the application of autologous platelet growth factors and vital neutrophilic leukocytes is gaining in popularity. However, more scientific
Evidence and data to support the use of platelet-leukocyte gel in clinical settings are mandatory in order to achieve progress in the use of these autologous biotechnology procedures. Recently, novel applications have emerged in the field of PLG applications and in a variety of surgical disciplines. We reviewed some of these new applications and report on some results of studies that indicate some potentially new directions and clinical applications.

Soft tissue healing and endoscopic applications

Promising results have been obtained in patients with chronic non-healing (diabetic) wounds after topical PLG application. Margolis et al. demonstrated that the application of the PLG was more effective than standard care methods in wound healing and that this treatment was even more effective in patients with deeper wounds (22). Furthermore, in our own experience we encountered improved wound healing when PLG was used during wound closure after total knee arthroplasty.

Soft tissue trauma, such as tendon and ligament ruptures and joint capsular injuries that occur frequently often require a surgical intervention. It is presumed that the combination of surgical repair with the application of biologically active PLG should accelerate healing with an improved outcome. Aspenberg and Virchenko showed, in an in-vivo rat model, that PLG applied to traumatized Achilles tendons increased tensile strength and stiffness by about 30% after the first week. The effect persisted for as long as 3 weeks after the injection, suggesting that the use of PLG in tendon repair with improved the physiological healing process.

Anterior cruciate reconstructive ligament surgery is routinely performed to reconstruct the ligament with an autologous graft. Since the procedure is mainly performed arthroscopically, it is therefore challenging to apply PLG to augment healing. Sanchez et al. reported enhanced healing with less complications and improved fixation of the graft within the bone tunnels in a retrospective clinical trial involving 100 patients.

The mechanism of action by which tendon repair is improved when PLG is applied, is based on release of vascular endothelial growth factor (VEGF). This growth factor stimulates angiogenesis, leading to an improved blood supply which is mandatory for the tendon repair process. Furthermore, released platelet growth factors induce a proliferation of tendon cells and stimulate production of VEGF and hepatocyte growth factor (HGF), a potent antifibrotic agent. The latter may be of importance in reducing scar formation and fibrosis in newly reconstructed tendon tissue, which might lead to poor outcomes.
In the past, fibrin glues have been used to make a suture less intestine anastomosis and for the treatment of gastrointestinal anastomotic leaks during laparoscopic surgery\textsuperscript{30,31}.

Recently, a novel PLG application has emerged in morbidly obese patients who underwent bariatric surgery. Brady \textit{et al.} used PLG, via an endoscopic delivery system (Figure 2), after a laparoscopic Roux-en Y gastric bypass procedure, in order to avoid hemorrhage, infection and anastomotic leaks, which may occasionally lead to death\textsuperscript{32}.

They suggest that the use of PLG contributed to an enhanced hemostatic response, accelerated tissue healing, improved collagen synthesis and thus

\textbf{Figure 2.} Aerosol controlled laparoscopic platelet-leukocyte gel delivery system. (Micromedics Inc, St. Paul MN, USA)
prevented anastomotic leaks, thereby improving outcome. Recently, Pomerantz and Dutton applied PLG during endoscopic sinus surgery in order to improve their packing technique. Their quality of life scores showed an improvement with regard to postoperative epistaxis, synechia formation, and exuberant granulation tissue in PLG treated patients.

**Synthetic and allogeneic implants**

Synthetic meshes and allogeneic implants are currently used in “tension-free” hernioplasty of the abdominal wall and in inguinal hernia repair. However, following cicatricial hernia repair serious complications have been observed (i.e. seromata, dense adhesions, and fistulization). Furthermore, Tyrell et al. have observed that the tensile strength of implanted meshes was markedly reduced due to absorption of the mesh. Lichtenstein hernioplasty is an accepted method for inguinal hernia repair. One of the most frequent complications which occur after inguinal hernia surgery is post-operative pain which, at times, is chronic and permanent and leads to a poor quality of life. Innovative tools to decrease the complication related to anchoring the mesh in position, include the use of tissue adhesives. Another option to secure the synthetic implants might be realized by applying PLG to the meshes instead of full suture lines. Autologous platelet growth factors have been used by Zieren et al. who showed an enhanced ingrowth and increased cell proliferation with a higher number of fibroblasts/collagen fibers in abdominal hernia repair, suggesting a role of platelet growth factors in the healing process. Sclafani et al. showed an accelerated maturation of wounds in an experimental setting when PLG was used. Based upon these findings, we have initiated a research program to confirm whether the application of PLG is useful in a clinical setting for hernia repair. The study objective is to determine the advantages of its use compared to current techniques, especially in minimizing pain after conventional and laparoscopic hernioplasties.

**Bone growth**

Impaired bone healing following fractures, with the development of pseudarthrosis, or fusion operations in case of nonunions, causes pain and disability. Attempts are being made to create bone substitutes but also technologies are being developed that improve bone healing by adding biological materials, such as PLG, to stimulate osteogenesis and osteoconduction. In bone healing (i.e. callus formation) platelets act as an exogenous source of growth factors stimulating the activity of bone cells, based on a unique role in bone growth. Numerous *in-vitro* studies have been performed using platelet gels. Since the
initial description of PLG use in maxillofacial surgery, subsequent studies have focused on the effect on a variety of bone-derived cells, including osteoblasts, osteoclasts, periodontal ligament cells, and mesenchymal cells. Gruber et al. demonstrated that proliferation of bone-derived cells was augmented, in a dose-dependent manner, when PLG was used. They suggested that, aside from a mitogenic effect, PLG application at the time of surgery enhances the bone healing capacity due to bone resorption and remodeling43. Another therapeutic application of PLG involves the combination of PLG with different bone substitutes. Several authors have used histomorphometric analysis to demonstrate a beneficial effect of PLG, when different bone matrices were used. Aghaloo et al. used natural de-proteinized bovine matrix, and measured an improved bone growth when this was used with PLG44. Suba et al. used tricalcium phosphate in combination with PLG and found more intense bone regeneration45. Furthermore, due to the sticky structure of PLG, caused by the fibrin strands present in the gel, bone substitutes are kept together, avoiding unwanted migration of bone particles. Recently, the percutaneous application of PLG in a diabetic femur fracture model was described. Normalized cellular proliferation and chondrogenesis, with an improved mechanical strength, was observed when PLG was injected in this model46.

Tissue engineering
A variety of methods have been used for the restoration of bone or soft tissue defects, in a different surgical settings, including orthopedic surgery, maxillofacial surgery and reconstructive surgery47,48. However, the manipulation and reinforcement of biocompatible materials in surgery is not always easy to achieve. Mixtures of autologous tissues have been used to accomplish restoration of defects. Tissue engineering is a technology that involves the morphogenesis of new tissues using isolated cells with biocompatible matrices and is often combined with growth factors. Mesenchymal stem cells (MSC) are multipotent cells which can replicate as undifferentiated cells with the possibility of differentiating into mesenchymal tissues (i.e. bone, cartilage, tendon, and muscle). This ability has made MSC a potential component of tissue engineering concepts. Recently, several research groups have been studying PLG as a matrix for tissue engineering models, since the activated PLG will release numerous platelet derived growth factors49,50. Another advantage of using MSC and PLG is that these two components are autologous, non-toxic, and biodegradable, proportional to the development of new bone formation when compared to allogeneic matrices which are non-degradable during the first weeks after implantation51.
Yamada et al. demonstrated in dogs, that the combination of MSC and PLG resulted in a significantly higher maturation of bone and neovascularization when compared to controls52. Thereafter, the same group used a combination of MSC and PLG for successful clinical alveolar bone augmentation47. The clinical application of PLG with bone marrow derived MSC was also reported by Kito in the course of distraction osteogenesis53. In soft tissue engineering, Anitua and co-workers identified the role of PLG releasate on cultured tendon cells. They state that the treatment of tendon injuries may be of benefit, since cell proliferation is induced with the promotion of endogenous angiogenic growth factor synthesis54.

Infection prevention

Many investigators focused at the exogenous application of concentrated and activated platelets in PLG in a variety of procedures that result in the material adhering to tissues and therefore initiating and accelerating wound healing17,22. However, in addition to the platelet gel delivery of growth factors, limited data are available addressing the role of leukocytes, present in PLG, to act as an antimicrobial component10. From our own experience we reported that P-LRP not only consists of a high concentration of platelets containing platelet growth factors but is also abundant in concentrated leukocytes, neutrophils, monocytes, and lymphocytes11. Neutrophils and monocytes are rich in granules containing, myeloperoxidase which catalyzes the oxidation of chloride to generate hypochlorous acid and other reactive oxygen derivates that act as potent bactericidal oxidants, which are toxic to micro-organisms and fungi55,56. Furthermore, Yeaman et al. and Tang et al. support the idea that platelets are also involved in microbicidal activity, and suggest that they play a role in the platelet host defense mechanism by releasing a variety of platelet microbicidal proteins57,58. The platelet microbicidal proteins were shown to be released after platelet activation, demonstrating potent activities against pathogens that have a tendency to enter the blood stream59.

Furthermore, we expect that exogenous PLG injections, rather than peri-articular injections with corticosteroids or even surgery, will be indicated to treat tendonitis and periarthrits. Such a therapeutic approach was cautiously suggested in an equine study in which PLG was applied to injured tendons60.
CONCLUSION

Platelet-leukocyte gels may become an ideal autologous prepared biological blood derived product, which can be exogenously applied to a diversity of tissues where it releases high concentrations of platelet growth factors that enhance healing. In addition it possesses antimicrobial properties that may contribute to the prevention of infections.

The current review suggests that the use of PLG may be beneficial in surgery. Platelet-leukocyte gels have been successfully used in maxillofacial surgery, orthopedics, cosmetic surgery and dental implantology. However, the procedure to prepare PLG and application techniques is likely to differ greatly amongst clinicians, resulting in inconsistent results. To avoid conflicting data, standardization of P-LRP methodology is therefore warranted. Furthermore, randomized controlled clinical trials are needed to study the effect of platelet-leukocyte gel on wound rehabilitation, functional recovery and on the promotion of bone growth. The bactericidal effect should be clarified and the role in tissue engineering should be defined. Overall one may conclude that the structures present in platelet-leukocyte gel appear to have a major beneficial therapeutic effect in surgery.
chapter 6
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