



THE UNIVERSITY OF MEDICINE AND PHARMACY
OF CRAIOVA
DOCTORAL SCHOOL

PHD THESIS

Abstract

THE FULL THICKNESS SKIN GRAFT ENHANCED WITH PLATELET RICH FIBRIN

Scientific coordinator

Prof. Univ. Dr. Marius Eugen CIUREA

PhD Student

Oliviu NICA



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Introduction

The treatment of wounds and burns is an important problem of plastic surgery because of their high incidence.

Skin grafting is the simplest and most effective method used in plastic and reconstructive surgery to cover skin defects. Often, however, complications can severely imperil the evolution of burned patients. Moreover, injuries located around the joints can cause severe functional impairment.

This study aims to verify the potential benefit of Platelet-Rich Fibrin in improving the speed and rate of graft integration, the reduction of complication rates and the improvement of functional and aesthetic outcomes. Accelerated wound healing leads to a reduced level of morbidity and shortens hospitalization, thus improving the quality of life for patients.

The data collected in the study was utilized in a comparative analysis between the control group skin grafts and the test group PRF treated skin grafts, in order to highlight the possible differences in healing.

The aims of this project were to obtain new data, relevant for both scientific research and clinical practice, data which could be applied to enhance wound healing and patient management.

Current state of knowledge

Skin grafting is the simplest and most effective method for covering skin defects used in reconstructive plastic surgery for skin defect management. Often, however, complications such as lack of skin graft integration or the development of hypertrophic scars can severely jeopardize the outcome of burned patients.

Platelet-rich Plasma (PRP) is a concentrated derivative of whole blood that is rich in platelets and plasma proteins. Due to its higher concentration of growth factors compared to circulating blood, it has been used by several surgical specialties to accelerate healing.



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Platelet-rich Fibrin (PRF) is a second-generation biomaterial; it is made up of a resorbable platelet-rich fibrin membrane that allows the gradual release of cytokines and growth factors.

The method is useful, minimally invasive (requires removing a negligible quantity of blood) and cost-efficient.

PRF could potentially be used to treat burns but also other skin defects.

This study focused on wounds with large skin defects, which were surgically treated with full thickness skin grafts augmented with Platelet-rich Fibrin (PRF).

For this study, wounds with skin defects were performed on the dorsum of Wistar rats. The lesions for the test group were covered with PRF and full-thickness skin grafts. The quality of the skin grafts was evaluated both clinically and histologically, and the results were compared with the control group.

Due to the fact that the healing process is imperfect, scarring continues to be a major clinical problem. Even if significant progress has been made in understanding the healing process, many fundamental issues remain unresolved.

Given the high number of patients who require skin grafts for the treatment of burns and other skin defects, the possibility of improving the operating protocol, by accelerating and improving the healing process and reducing the occurrence of complications, underlines the need for this study. Treatment accessibility, the speed and ease of implementation, the fact that it is minimally invasive, and the low costs further outline the importance of the study.

The wound healing process has been widely studied in the last century, being well understood and characterized in great detail both physiologically and histologically.

The skin is the largest organ of the body and has numerous roles. Wound healing exhibits an extraordinary mechanism of cascading events, that is unique in nature. It is performed in several essential steps in order to repair the integrity of the skin and to restore its function as a protective barrier.

Any alteration of the normal chemotactic signals, at any stage, can produce defective wound healing.



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Wound healing is a fundamental and complex physiological process, important for maintaining homeostasis and combating infection. This process relies on a finely tuned collaboration of many types of cells and their products.

The healing process can be divided into four predictable, partially overlapping and succeeding stages: hemostasis, inflammation, proliferation and tissue remodeling.

For a wound to heal successfully, all four phases must appear in the proper sequence and time interval. Many factors can interfere with one or more phases of this process, thus causing defective healing.

Platelets induce primary hemostasis by adherence and aggregation at the site of the lesion and trigger secondary hemostasis through the activation of the coagulation cascade.

The contribution of platelets to the normal function of the vascular system and their role in blood vessel repair is well known and amply described in the literature. However, platelets are much more than just another component of the hemostatic system.

New studies have detailed their role as part of the tissue repair systems, having an important role in initiating the inflammatory process and stimulating the immune system.

To fulfill these roles, platelets must act as an integrated part of the immune system, synchronizing with leukocytes and other elements.

Normally, platelets are found in an inactive, nonadhesive state as they circulate within the blood stream. This state is maintained with the help of nitric oxide and PGI₂ secreted by the uninjured endothelial tissue. If the endothelial cells are damaged as a result of an injury, the platelet activation process is initiated, and they are subsequently recruited as part of the inflammatory response.

Platelet activation begins seconds after adhesion occurs and is critical for initiating and sustaining hemostasis. It is triggered when the sub-endothelial collagen is exposed and binds to the receptors found on the surface of platelets.

Activation allows platelets to degranulate and release chemotactic agents, growth factors, proteases and vasoactive agents (serotonin, histamine).



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Chemokines released by platelet activation attract inflammatory cells to the area, which, in turn, causes a transition to the next phase of the healing process – the inflammatory stage.

As part of the inflammatory response, platelets release numerous growth factors – PDGF, FGF- β , TGF- β , PDAF, VEGF, EGF, IGF, IL-8 and fibronectin. Of these factors, PDGF is considered the most important for the tissue repair process, due to its impact on angiogenesis, as well as fibroblast proliferation. There are numerous studies which have assessed the application of platelet rich biomaterials to promote wound healing.

After the activation of platelets trapped in the fibrin matrix, numerous growth factors are released. These, in turn, stimulate a variety of other elements involved in tissue healing. Over the past two decades, the expanded understanding of the physiological roles platelets play in wound healing, has led to the development of several platelet-derived biomaterials for various therapeutic applications.

Repair of damaged tissue begins very early with a regulated sequence of biochemical events set in motion to repair the damaged. Although imperfect, these processes usually perform as per their biological programming leading to wound closure and tissue remodeling.

As mentioned before the healing process is usually divided into four predictable phases, which may overlap: hemostasis, inflammation, proliferation and tissue remodeling. While the understanding of this whole process is still incomplete, it has been previously established that platelets play a decisive role not only in hemostasis, but also in wound healing, through an abundance of growth factors and other signaling cytokines which modulate the inflammatory response. Historically it has been proven that fibrin plays a key role in the adherence of biological dressings and autografts, its presence being associated with a higher rate of graft adhesion.

To this end, we evaluated the role of a platelet-rich biomaterial – platelet-rich fibrin – in the augmentation of full-thickness skin grafts.

The histological similarities between rat and human skin, prompted the selection of the Wistar rat as the experimental animal model, as it would produce scientifically accurate results. Adequate living conditions were provided for the Wistar rats by the animal laboratory staff.



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Personal contributions

The study included 40 male Wistar rats provided by the University of Medicine and Pharmacy Craiova.

The rats were anesthetized by an intraperitoneal injection of a mixed solution of Ketamine and Xylazine. We opted for a rectangular is 3×2 centimeters lesion on the dorsum.

Incisions were performed on the outlined preoperative marks. The skin was detached from the deep muscular fascia and harvested.

The graft was trimmed of all underlying tissues. The subcutaneous panniculus carnosus muscle was also dissected from the dermis, in order to simulate a full thickness skin graft.

The skin graft was then secured using a simple interrupted 4-0 Polypropylene surgical suture.

The same surgical procedure was performed on the test group, with the only difference consisting in the application of the prepared PRF to the deep muscular fascia bed.

The healing process was assessed daily and, on the 21st postoperative day, the rats were euthanized, and their full thickness skin grafts were harvested for histological and immunohistochemical analysis.

The skin grafts were evaluated macroscopically using planimetry. Skin graft assessment was conducted by calculating the area of dermal necrosis as a percentage of the total surface area in the skin graft sample.

The dermal necrosis rate of the full thickness skin graft was compared between the control group and test group.

The average percentage of necrosis in full thickness skin grafts, augmented with PRF (14.9%, SD=5.1) was significantly lower than in the control grafts (28.5%, SD=9.2) – $p<0.01$.

The histological examination quantified changes of the epidermis, the presence of fibroblasts and the number of blood vessels.



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The average thickness of the epidermis of the full thickness skin graft was compared between the test and control groups. The 35% difference between the average thickness of the epidermal layer (not including the stratum corneum) was eloquently high $p < 0.01$. Also, a significant difference was observed in the standard deviation of the control group, as opposed to the test group, objectifying the vast differences in epidermal thickness identified in the histological samples from the control group.

The average fibroblasts and fibrocytes count per slide in the dermal layer of the full thickness skin grafts augmented with PRF (test group) was significantly higher than in the control group $p < 0.01$. The 41% difference also translated in a greater number of myofibroblasts in test group.

Blood vessels were identified using low power microscopy. The prominent vascular areas were then studied in higher power fields; individual vessels were manually marked and counted. Vascular density was calculated using ImageJ's Fiji Vascular Density plugin. Subsequently, the average number of blood vessels was calculated for each slide. We found an increase in the number of blood vessels and the vascular density in the test group slides, but it provided weak evidence against the null hypothesis – a 6.74% difference in the of blood vessel count and 24.8 % difference in vascular density measurements – $p > 0.05$.

The epidermal layer in the test group was thicker, more uniform and had complete cohesion with the dermis, compared to the skin of the control group. This could be explained by the improved adhesion of the graft provided by fibrin, which could limit necrosis in the superficial dermal layers during the plasma imbibition stage, as well as providing a scaffold that promotes capillary growth in the fibrin matrix.

Recent studies have confirmed that an increase in the number of fibroblasts has been associated with accelerated wound healing.

The origin of fibroblasts in skin grafts remains ambiguous. Some authors have hypothesized that these cells migrate from the blood, while other sources theorize that the origin is in the local perivascular mesenchymal cells. Whatever the case, most authors agree that fibroblasts are not indigenous and migrate from healthy surrounding tissue through chemotactic signaling.



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The strong chemotactic effect of PDGF on fibroblasts has previously proven, with PDGF causing fibroblasts to migrate to the wound site but also inducing proliferation. The affluence of growth factors could explain the higher number of fibroblasts and myofibroblasts in the test group, which, in turn, would support the observed accelerated healing process.

While the microvascular density analysis showed an increase in the number and caliber of the blood vessels in the dermis of PRF augmented skin grafts, it provided insufficient evidence to confidently reject the null hypothesis. However, it has been previously demonstrated by other authors that the well-known angiogenic and vasculogenic properties of VEGF may not have a major effect on the actual number of vessels. Furthermore, VEGF also influences vascular permeability, which may further explain the lower percentage of dermal necrosis and faster epithelialization of PRF-treated grafts. The results could also be attributed to the increase in blood vessel size and the growth of newly formed blood vessels in the wound bed, in addition to the transplanted tissue. Although we could not demonstrate the angiogenic capabilities of PRF, a reduced degree of dermal necrosis was observed in rats treated with PRF.

The biological activity of fibrin, produced through the slow polymerization of fibrinogen, has been established as an extremely important stimulating factor for the healing process, providing the necessary scaffold for cell adhesion and migration.

Conclusions

With its rich cellular component and the release of growth factors, the biomaterial presents all the attributes necessary to demonstrate its significant healing capability, also certified in previous studies and supported by this one.

Our limited data supports the theory that adding PRF to the wound beds intended for grafting, has the potential to accelerate the healing rate, the ability to improve graft adherence and to regulate the proliferation of a thicker, more uniform epidermis, while reducing dermal necrosis and the duration of the epithelialization process.

Even though further studies are necessary to reveal the full potential of PRF and improve its use, the strategies for accelerating translational research, make biological scaffoldings enriched with platelets, to have an increasingly higher use in medical practice.