This thesis explores the effect of platelet-rich plasma (PRP) on the healing of acute burns and the burn scar quality, and conversely the effects of burn injury on platelets. This final chapter summarizes the findings of this thesis, puts them in context with up-to-date literature, and reflects on clinical implications and future perspectives.

The main question of this thesis was: what is the attributive value of PRP for burn wound healing and consequently the quality of burn scars?

We tried to answer this question by studying the following sub-questions:

1. What is the current evidence from the literature on PRP and specifically on PRP in burns?
2. What is the effect of burns on the quantity, function and quality of platelets in burn patients?
3. What is the effect of burn injury on the quality of autologous PRP?
4. What is the clinical effect of PRP on burn wound healing and outcome of burn wounds?

CURRENT EVIDENCE FROM THE LITERATURE ON PRP

Background to PRP

Platelet-rich plasma (PRP) is a derivative of whole blood that contains a supra-physiological concentration of platelets. Platelets store growth factors in their alpha granules, which are released after activation.1 These growth factors are involved in the phases of wound healing (inflammation, proliferation and remodelling) in many ways, such as by promoting chemotaxis, cell adhesion, mitogenesis, proliferation and angiogenesis. The most studied of these factors are platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor (TGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF).2

The delivery of an abundance of these growth factors has shown positive effects on wound healing in in vitro analyses.3 There are also many studies advocating positive effects of PRP on wounds in many different forms. However, our review in chapter 2 highlights some of the difficulties in this field: variables exist in the preparation and application of PRP, making comparison and interpretation of the evidence far from easy.

Firstly, there are many different types of platelet-rich products with a broad nomenclature. There are not only different names for one similar product, but the products are actually often very different, with different preparation procedures and consequently different content, and they vary in fibrin composition and the presence of leukocytes and/or erythrocytes.

Especially the presence of leukocytes is the subject of on-going debate. Some authors advocate the use PRP without leukocytes because of their potential negative pro-inflammatory effects, and others claim antimicrobial effects of the leukocytes in PRP. A recent review of the subject summarized existing evidence and concluded that despite a number of studies showing that preparations that include leukocytes have antimicrobial properties, there is not enough evidence to attribute all these bactericidal effects to the presence of leukocytes alone. PRP preparations, with or without leukocytes, showed bacteriostatic characteristics against the majority of the bacterial strains tested.4 On the other hand, proper evidence of harmful effects of leukocytes on wound healing is lacking as well.

Apart from different names and content, there are also different activation and application methods. Finally, there are an inter-patient variable platelet baseline count and platelet yield
rate after PRP production, which also result in variable amounts of growth factors. In brief, a vast heterogeneity exists in PRP products, and the only unequivocal factor is that PRP has an increased number of platelets, which could effect the wound healing process due to its growth factors and cytokines being released after activation.

In January 2016, the Platelet Physiology Subcommittee of the International Society on Thrombosis and Haemostasis (SSC/ISTH) formed a working party of 10 experts (including the author of this thesis), whose task it was to produce a series of consensus recommendations for standardising the use of platelets in regenerative medicine. The working party used a formal consensus method (the RAND method) to develop final recommendations and produced an invited guideline entitled ‘Guidance on the use of platelet-derived biomaterials in regenerative medicine and proposal for a new classification system: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH’.5

This guideline proposes a new classification system based on the following: content (presence of leukocytes and erythrocytes, and fibrin composition); activation (use of activation, without activator, frozen-thawed preparations); platelet concentration (subdivided into three categories based on the platelet count (< 900 x 10^3/μl, 900 – 1 700 x 10^3/μl and > 1700 x 10^3/μl); and finally the preparation methods (gravitational centrifugation techniques, standard cell separators, autologous selective filtration technology (plateletpheresis) (Table 1). Development of these standardization and evidence-based guidelines and following the recommendations by researchers in the field are essential to the progress of PRP research.

Evidence from the literature on PRP in different applications

Chapter 2 summarizes the literature on the application of PRP in a number of different fields of medicine, such as maxillofacial surgery, orthopaedics and sports medicine, chronic wounds and aesthetic surgery. The overall conclusions were that there are positive as well as conflicting reports based on low-quality studies with many uncertainties on the quality and application of PRP, making general and definite conclusions virtually impossible. As Borzini stated in 2007: ‘From a scientific viewpoint, a vast majority of positive papers are not proofs for platelet gel to be considered fully effective, science is not democracy.’6

Some systematic reviews and new trials have recently been published. A recent Cochrane review on the application of PRP in the wound care of chronic wounds included 10 randomised clinical trials, with a total of 442 participants (mean age 61 years and 42% women). Autologous PRP increased the healing of foot ulcers in people with diabetes compared with standard care, but it was unclear if autologous PRP had an effect on other types of chronic wounds. These findings are based on low-quality evidence due to the small number of studies and patients included, and their poor methodological quality.7

In aesthetic medicine PRP applications have greatly taken flight, especially infiltrating PRP in the face, the so-called ‘vampire facelift’. However, again robust evidence of effects is scant. A recent randomized, double-blind, placebo-controlled study on the addition of PRP to lipofilling in the face showed that it significantly reduced postoperative recovery time but did not improve patient
Table 1. is the proposed new platelet-rich plasma (PRP) classification system table from recent publication Harrison et al. \(^5\)

<table>
<thead>
<tr>
<th>Class</th>
<th>Leukocytes + &gt;= 1%</th>
<th>Red Cells + &gt;= 10%</th>
<th>Fibrinogen/ Fibrin Activation</th>
<th>Platelet concentration</th>
<th>Preparation category</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>-</td>
<td>Low</td>
<td>I</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>Red-PRP</td>
<td>+</td>
<td>-</td>
<td>III</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>L-PRP</td>
<td>+</td>
<td>-</td>
<td>Low</td>
<td>I</td>
<td>A 1</td>
</tr>
<tr>
<td>Red-L-PRP</td>
<td>-</td>
<td>+</td>
<td>III</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>PRF</td>
<td>-</td>
<td>High</td>
<td>I</td>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>Red-PRF</td>
<td>+</td>
<td>-</td>
<td>III</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>L-PRF</td>
<td>+</td>
<td>-</td>
<td>High</td>
<td>I</td>
<td>A 1</td>
</tr>
<tr>
<td>Red-L-PRF</td>
<td>+</td>
<td>-</td>
<td>III</td>
<td>C</td>
<td>3</td>
</tr>
</tbody>
</table>

Activation is divided into three subcategories:
I for the use of activation
II for PRP application without activator
III for use of frozen-thawed preparations

Platelet concentrates are subdivided into three categories (A, B and C) based on the platelet count range in the samples.
The three categories are:
A Platelet count < 900 x10^3 /μl
B Platelet count 900 – 1700 x10^3 /μl
C Platelet count > 1700 x10^3 /μl

The preparation methods are classified into three categories:
1 The Gravitational centrifugation techniques
2 Standard cell separators
3 Autologous selective filtration technology (plateletpheresis).

Abbreviations:
L-PRF, leukocyte-rich platelet-rich fibrin; L-PRP, leukocyte-rich PRP; PRF, platelet-rich fibrin; Red-L-PRF, red blood cell-rich and leukocyte rich platelet-rich fibrin; Red-L-PRP, red blood cell-rich and leukocyte-rich PRP; Red-PRF, red blood cell-rich platelet-rich fibrin; Red-PRP, red blood cell-rich PRP.

+ or _ defines whether (+) or not (-) there are leukocytes equal or greater than (=) 1% and/or Red cells equal or greater than (=>) 10% of the total cell counts (including leukocytes, platelets and Red cells) in the PRP.

outcome in terms of skin elasticity, improvement of the nasolabial fold, or patient satisfaction.\(^8\)
The authors did not report any information on the quality of the PRP itself.

A summary of all recent relevant evidence in sports and orthopaedics falls beyond the scope of this thesis; however, the general conclusion can be drawn that there are positive and inconclusive reports, with a general lack of strength of evidence.\(^9\) For example, Grassi et al. performed a thorough meta-analysis on the application of PRP in acute muscle injuries. They concluded that although PRP treatment was a safe procedure as the studies reported only negligible adverse effects, in terms of efficacy the current literature did not support its use for muscle injuries.\(^10\) However, the lack of robust
evidence has not stopped the great increase in the use of PRP for musculoskeletal indications, which is partially explained by the promising in vitro results, low regulatory issues and popularity fuelled by celebrity testimonials from athletes such as Tiger Woods and Rafael Nadal. As a consequence many clinical trials have been started, unfortunately often without full understanding of all aspects of PRP, possibly leading to disappointing results. As Murray outlines so well: ‘PRP may ultimately be considered a hype, however there is also a danger that a potentially beneficial treatment is dismissed as non-effective simply because suboptimised preparations were used in these studies. We will only truly know if PRP can be of therapeutic benefit if the scientific/clinical community accepts that shortcuts cannot be taken, and adopts a comprehensive ‘back to basics’ approach.’

**Evidence for the application of PRP in burns**

The literature on the use of PRP in burns was systematically searched in chapter 2. We concluded that the evidence was very limited, ranging from some animal studies to case reports to small patient cohorts. Long-term outcome in this field is even more limited: only one study showed accelerated recovery of the viscoelastic properties of full-thickness burns treated with autologous platelet concentrate with a SSG, compared with areas treated with a SSG alone, but no improvement in the long-term outcome was shown.

Theoretically, a deep dermal burn could benefit from PRP in several ways. First, the haemostatic qualities of PRP could increase the take rate of the skin grafts by decreasing continued bleeding, working as a fibrin glue, as well as providing a well-vascularised bed for the meshed skin graft. Next, PRP could exert a stimulating effect on wound healing by a growth factor-mediated contribution to faster wound closure of mesh interstices. However, a burn injury has a different physiology than chronic or acute injuries, and moreover burn patients are in an altered systemic physiological state, where platelets already seem to be activated. This could influence the quality of the platelets in PRP compared to the healthy subjects in whom PRP was mostly used. It was established in chapter 2 that no information could be retrieved on exactly how burn injury affects platelets and how this could influence the effects of PRP used in burn patients.

**GENERAL CONCLUSION AND FUTURE PERSPECTIVES ON CLINICAL USE OF PRP IN GENERAL**

To return to the aims set out at the beginning of this thesis, we investigated what PRP is and explored its mechanisms of action. Furthermore we reflected on the current evidence for the different indications in which PRP is used. We established that a huge variation in PRP products and effectiveness exists. We advocate (in accordance with the working party of the platelet physiology subcommittee of the SSC/ISTH in which the author of this thesis is a participant) that future research should focus on assessing the functionality of PRP in a standardised way and that in future trials the quality and content of the PRP should be taken into account and this should be correlated with clinical outcome.

Next we evaluated the existing evidence on the use of PRP in burn injury treatment and established that the evidence is limited; however, we can assume that in theory a burn injury could benefit from treatment with PRP. We realised that the systemic effects of the burn injury could affect
platelets in burn patients. This brings us to the important sub-question of this thesis: what effect does burn injury have on platelets?

THE EFFECT OF BURNS ON THE QUANTITY, FUNCTION AND QUALITY OF PLATELETS IN BURN PATIENTS

Platelet quantity post-burn injury

In chapter 3 we performed retrospective quantitative analyses in 244 burn patients and found that platelet counts show a distinct time course post-burn injury with a nadir at day 3 and a peak at day 14, followed by a general return to normal. This seemingly simple observation has, however, never previously been confirmed in a large patient cohort. Furthermore, it was found that this time course is influenced by the percentage of total body surface area (%TBSA), age and sepsis and not by gender.

However, beyond this plain platelet count, it was found that a high %TBSA, higher age and low thrombocyte peak predicted mortality, and there were indications that a low nadir could also be associated with mortality. The incidence of sepsis was too small in our study group to perform a reliable Cox regression analysis; therefore we could not assess the predictive value of thrombocytopenia for sepsis. This would be very interesting, since platelets are, besides being involved in haemostasis, also important modulators of the host response during infection, serving as sentinels in our circulatory system. Whether thrombocytopenia represents platelet activation and consumption as a primary pathologic event or simply serves as an indicator of disease severity is unknown.

Since then, several recent studies have elaborated on our findings. In a larger subgroup of 145 burn patients with a TBSA greater than 20%, Cato et al. confirmed our findings on the pattern of platelet counts post-burn injury. Moreover, the platelet count at the nadir (day 3) combined with %TBSA had a modest association with sepsis. Low peak platelet count showed a strong predictive power for mortality in a multivariable model with TBSA, age and the revised Baux score. Dinsdale et al. continued with this in a recent scientific report in Nature, where they analysed 39 burned patients (TBSA 15–39%) with the Sysmex XN-1000 measuring three methods of platelet counting in parallel (namely impedance, optical and the more accurate fluorescent count). They wanted to establish if thermal injury caused direct damage to circulating red blood cells that can result in red cell fragmentation, with the appearance of large numbers of microspherocytes causing overestimation of platelet counts by commonly used impedance analysers. In the Burn Centre of Beverwijk, the laboratory staff regularly verifies haematology results microscopically and did not see cases of spurious platelet counts. Dinsdale et al. confirmed this and did not see a difference between impedance, optical and fluorescent platelet counts. They corroborated the finding that platelet counts had a nadir at day 3, followed by a rebound thrombocytosis at day 21. Furthermore, they showed nadir values to be significantly lower in septic patients. Qiu et al. found in another recent retrospective analysis of a series of 610 burn patients that the red blood cell distribution (RDW) to platelet count (PLT) ratio (RDW-to-PLT ratio, which they call RPR) values on the 3rd and 7th days were significantly associated with the mortality rates of severe burn patients (P < 0.01).
claim that the RDW-to-PLT ratio could serve as an independent and novel marker for mortality rate prediction in severe burn patients.19

**Function and quality of platelets post-burn injury**

To evaluate the effects of burn injury on platelets, the activation, function and content of platelets was studied in chapter 4. In six burn patients with more than 15% TBSA burned, blood samples were collected at five designated time points post-burn: day 0–1, day 3–4, day 8–9, day 12–16, and day 20–24, corresponding respectively to the burn day, the nadir, the rise, the peak and the return to normal of the platelet count. Several platelet function, activation and growth factor quantification analyses were performed. It was found that platelets post-burn injury appeared to be functional and not overly activated. However, the burn patients seemed to remain in a procoagulant state for an extensive period of time post-burn.

This chapter engaged with another interesting and complex field on its own: the coagulation process, in which platelets play an important role. Coagulopathy is not strictly defined and a high heterogeneity exists in the literature on coagulopathy in burns. A wide range of diagnostic criteria for, and definitions of, coagulopathy have been used, which makes it difficult to draw overall conclusions. However, with knowledge evolving and the emergence of new tests, there is nowadays more insight into the coagulation process in burn patients. Conventional coagulation assays, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) are often elevated, indicating a tendency to bleed; however, these assessments only reflect a part of the coagulation process. More advanced viscoelastic coagulation assays (such as the TEG used in chapter 4) mirror the clotting process in vivo, where all cellular blood components involved (besides the endothelial factor) contribute to the clotting process.20 In trauma patients fibrinogen deficit and hyperfibrinolysis are the leading pathomechanisms of bleeding; however, this is not the case in burn victims, as shown in Chapter 4, where fibrinogen was found to be increased. This is in accordance with other studies, which was confirmed for the first time for a period longer than one week post-burn injury.20 Potentially, burn patients rebalance their fibrinolytic system during the post-burn time course, since it was shown in another study that after burn and inhalation injury, patients showed an antifibrinolytic shift seven days post-burn.21,22 Since we have found procoagulant changes up to 24 days post-burn, it will be interesting to determine the fibrinolytic status for this extended period post-burn, which will allow us to obtain a more comprehensive understanding of the full coagulation process post-burn injury and highlight potential clinical implications.

Furthermore, we established in this chapter that growth factor content followed the same course as the platelet count, reflecting a constant growth factor per platelet ratio.

This implies that for the sake of the production of PRP and the wish for a multitude of growth factors, it would be advisable to produce autologous PRP beyond the nadir period to increase the yield of platelets and hence growth factors.

**Conclusions and future perspectives on platelets post-burn injury**

In view of our initial aim to gain more information on the effect of burn injury on platelet quality, quantity and function set out at the beginning of this thesis, we can conclude that the time course
of platelet count post-burn injury brought to light by our research in chapter 3 now seems well established and has been confirmed by numerous other authors. As an interesting aside, it was found that platelet counts offer predictive value of mortality and sepsis. The question is how to incorporate this in daily care. Although no rigid cut-off values are offered just yet, since platelet counts are routinely available they can be interpreted. A very low thrombocytopenia around day 3, or a persistent thrombocytic period, or a decreased thrombocytic period could raise awareness among burn clinicians that the patient is at risk for sepsis or mortality. Future research in larger cohorts could throw more light on the relationship between platelet count and sepsis. Since sepsis is always a risk for burn patients, it would be very valuable to have a proper prognostic marker for this – platelets have been shown to play an essential role in this process. 

Regarding the qualitative effects of burn injury on platelets, we found that platelets post-burn injury appear to be functional, not overly activated and not deprived of growth factors. However, burn patients seem to remain in a procoagulant state for an extensive period after the burn injury has been inflicted. Further research is necessary in a larger patient cohort to clarify the whole coagulation process in burn patients, including the potential fibrinolytic process. This will be a complicated undertaking, since coagulation is such a multifaceted process, especially in a multi-variable group such as burn patients with different burn sizes who are undergoing resuscitation protocols, repetitive surgery, and blood and plasma transfusions and who are at high risk of infection and sepsis. However, it will be very valuable and will add essential information to our understanding of coagulation post-burn and how to treat patients optimally, balancing pro- and anti-thrombotic management objectives.

THE EFFECT OF BURN INJURY ON THE QUALITY OF AUTOLOGOUS PRP

The quality of PRP of burn patients

Returning to the main goal of this thesis with our sub-question on the systemic effects of burn injury on platelets in mind, we asked whether the quality of autologous PRP obtained in burn patients was satisfactory. We therefore performed growth factor quantification of the autologous PRP of burn patients and compared this to the autologous PRP of healthy volunteers. For this study we used a commercial ‘point of care’ system (GPS III, Biomet), which is often used both in daily practice as well as in several clinical studies. Chapter 5 describes the results of growth factor quantification analyses of the leukocyte-rich PRP of five burn patients, compared to the same type of PRP from five gender and age-matched healthy volunteers.

It was discovered that the PRP of burn patients has comparable levels of growth factors compared to the PRP of healthy volunteers. However, a considerable intra-individual variation in growth factor content was noted, which is in agreement with findings in the literature. A clear correlation was found between platelet count in PRP and most of the growth factors measured. This implies that measuring the platelet count in the PRP is an adequate and far more feasible quality check of PRP in daily practice as well as in a study setup. This is also advised by the working party of the platelet physiology subcommittee of the ISTH mentioned above.
Conclusions and future perspectives on PRP quality

An important point to be made is that the ideal platelet concentration (and hence the optimum quantity of growth factors) is not known for any indication. Most authors state that at least $0.8\text{–}1 \times 10^6/\mu l$ platelets should be present in PRP; however, there is no proper evidence for this, and this is probably the most persistent and worst statement referred to in the PRP literature. We may refer to this statement as ‘parrot proof’, meaning that authors are merely mimicking each other as parrots do. If this statement is tracked to its source it leads back to an abstract from a poster presentation from 2002, and cannot be considered reliable evidence for compulsory platelet concentration. However, this number of at least $0.8\text{–}1 \times 10^6/\mu l$ platelets is now broadly used. It might also be incorrect to strive for a maximum of platelets in PRP, since a few in vitro analyses have indicated that too many platelets could have negative effects. Further in vitro analyses could provide us with more information on required concentrations; however, in vitro systems will always be a limited reflection of the complex processes in vivo. In vivo data is scarce. Recently Louis et al. performed a randomized, double-blind, non-inferiority trial, in which they compared PRP injections with hyaluronic acid (HA) in knee osteoarthritis, and correlated clinical outcome with growth factor analyses. They found comparable effects of HA and PRP at 3 months, but interestingly, they found a significant correlation between the doses of TGF-b1 and PDGF-AB and the worsening of pain scores at 3 months, indicating a negative effect of too high doses of growth factors. Ideally, future studies should routinely state their platelet counts or perform growth factor quantification of the applied PRP, which could give greater clarification on which concentrations may be suitable for different indications.

The Effect of PRP on Burn Wound Healing and Scar Quality

Clinical trial data

Finally, to answer the main question of this thesis in chapter 6, a randomized, double-blind, intra-patient controlled study on the application of platelet-rich plasma in the treatment of deep dermal burns was described. This trial investigated the effect of autologous (leukocyte-rich) PRP on primary wound healing by evaluating the take rates and re-epithelialization rates of acute burns treated with split skin grafts (SSG) in combination with PRP compared to SSGs only. Lastly, we evaluated the effect of PRP on scar quality until 12 months after intervention.

We did not find a statistically significant difference between the mean take rate nor the mean epithelialization rate at day 5–7 between the PRP-treated and standard treated areas. However, since our patient group was a representative clinical sample of burn patients treated in a burn centre, there was a wide range between primary outcomes, and we therefore dichotomised the outcomes. We then found that PRP-treated wound areas more often showed better or equal epithelialisation and take rates at day 5–7 than the standard treated areas. Minor positive effects of PRP were also seen in the re-operated and in the early operated (≤7 days) subgroups.

This last finding seems somewhat contradictory, since we discovered earlier that in this period in particular platelet counts are low and thus growth factor amounts are low as well. However, in
sub-analyses we did not find a correlation between the platelet count in PRP and outcome in take rate or epithelialisation rate, not even if we corrected for area treated with PRP, in other words concentration. An explanation for this finding could be that in this group (≤7 days) in general the take and epithelialization rates were lower compared to the patients who were operated on later, so especially in this seemingly vulnerable group PRP was shown to have a positive effect on graft take rate and epithelialization rate.

Since this RCT was done, no other studies have been published on PRP and burn wounds. Hersant et al. investigated whether SSG with autologous PRP could accelerate and improve wound healing after cutaneous reconstruction for tissue loss secondary to soft tissue infections (no burns). Twenty-seven patients were randomized into two groups. In the PRP group, patients had a significant improved take rate of the SSG of 90% vs. 77% in the control group. Time until complete healing was also significantly shorter in the PRP group compared to the control group: 37.9 days vs. 73.7 days. However, this study was small with no blinding or objective outcome parameters, and no quality control of the PRP was done.

An important feature of our RCT was the long follow-up, providing evidence of the scar quality of burn wounds treated with PRP. At 3, 6, and 12 months postoperatively, the POSAS scores of the patients from the observers’ measurements with the Dermaspectrometer and Cutometer did not show a significant difference between wounds treated with PRP and those treated with the standard method. This also means that the scars of PRP-treated wounds were no worse than those of standard treated burns. This is also an important finding, since some growth factors present in PRP (TGF-1, TGF-2, PDGF) are involved in hypertrophic and keloid scarring of both normal skin wounds and burn wounds. This finding is in accordance with the literature, where there are no reports of increased scarring in PRP-treated wounds; however, long follow-ups are scarce.

Conclusions on the effect of PRP on burn wound healing and scar quality

Does this RCT provide adequate answers to our primary question as to whether PRP aids burn wound healing, leading to less disfiguring scars? The answer is ambiguous. Although the RCT was performed thoroughly, in hindsight there were quite a few variables added to an already variable PRP study setup as mentioned in chapter 2. There were different sizes of burns treated with PRP, with different mesh sizes, at different times post-burn injury. Although we studied the effect of these variables in sub-analyses, it is hard to draw firm conclusions from them, since these sub-analyses are obviously not adequately powered. Although the study reflects a proper cross-section of a clinical burn patient population, it is possible that a positive effect in a sub-group was concealed by the considerable variation caused by all the variables, as could be indicated by the minor positive effects we found.

In retrospect it would have been better to construct a more standardised setup, in which correlation of platelet count in PRP with a standardised area would already rule out several variables and provide more information on optimal platelet count. Nonetheless, if PRP had made an actual substantial difference to wound healing and consequently scar quality, we would probably have been able to measure this. So in the setting of acute burns, the addition of autologous PRP to a burn wound with a SSG does not seem to be justified. On the other hand, PRP appears to have some
effect, although it seems to be minor. Therefore the idea of adding platelets to burn wounds should not be altogether discarded.

FUTURE PERSPECTIVES ON THE EFFECT OF PRP ON BURN WOUND HEALING AND SCAR QUALITY

To summarise, we know that platelets post-burn injury are functional, not overly activated, and not deprived of growth factors in whole blood nor in a PRP product; and we learned that the application of autologous PRP in acute burn wounds does not significantly improve the take rate and epithelialisation rate of SSG or burn scars, although it does seem to have a slight beneficial effect on the wound healing of acute burns.

How to proceed from the main findings of this thesis?

In order to reach progress in PRP research more standardization is essential. Nevertheless, patient variables, such as baseline platelet count and growth factor content, are difficult to control. Therefore it seems worthwhile to explore a shift towards allogeneic PRP. This has several theoretical advantages. Preparing allogeneic PRP would eliminate the burden of drawing blood from a patient, sometimes more than 60 ml for a single application of 6 ml PRP. In blood banks, platelets are stored in abundance, since platelets are a side-product of blood donations. Donor blood is mostly used for its erythrocytes, given to patients in packed cell transfusions. Blood donors are thoroughly tested and blood transfusions are considered to be relatively safe, although allergic reactions and transfusion-transmitted infections cannot be completely ruled out. Not much is known about the safety of topical application of blood products; however, it can be expected that the risk for potential reactions would be lower than it would be after systemic administration. A recent study analysed the local immunological effects of allogeneic PRP in in vitro models. The study found that allogeneic PRP can promote the differentiation of monocytes to a regulatory anti-inflammatory population, possibly favouring wound healing. However, it was not clear if allogeneic PRP either matched or mis-matched for AB0 and Rh antigens could lead to different responses. Nevertheless, the authors suggested that the drawback of an immune response typical of allogeneic molecules used in a therapeutic setting would be either not relevant or even negligible.

In the current literature several case reports and case series have been published on the use of allogeneic PRP in wound care. None of them have reported adverse effects. An Italian group reported the outcomes of 115 patients with soft tissue loss in the fingers after trauma who were treated with allogeneic platelet gel twice weekly. The authors stated that they had used this type of gel for years in all sorts of wounds. Furthermore, there is one trial from Iran of 50 burn patients treated with allogeneic PRP compared to treatment with silversulphadiazine in an intra-patient controlled manner, with positive effects of allogeneic PRP on epithelialisation and no adverse events; however, the methodological quality of this report should be regarded with reservation.

Another advantage of allogeneic PRP is that it offers more standardization and can be custom made, thus eliminating many of the variables described in chapter 2. Its wide availability also makes it easier to test repeated applications of PRP.
Another new development is to produce PRP as a powder by means of lyophilisation (freeze-drying). A recent study found consistent growth factor concentrations in a PRP powder made by pooling and processing allogeneic platelet concentrates, after which resuspension with saline chloride provided approximately 1,000 identical “classic” PRP applications of 3 mL each, which could be stored for a year. A lyophilised PRP powder has even been tested in burn wounds in a recent trial in Taiwan. Lyophilised PRP powder was applied to deep second-degree burn wounds every 4 days and compared with an unspecified placebo. The authors found significant increased wound closure after 3 weeks of 93% in the PRP powder group compared to 85% in the control group. However, there are serious methodological issues: the placebo was not specified, the study was not blinded, the applications were performed on so-called deep second-degree burns, which are usually treated with excision and split skin graft. Importantly, there was no mention of the allogeneic nature of this product, nor were adverse effects encountered or safety issues reported.

In conclusion, this thesis is a journey through the world of platelet-rich plasma, wound healing and in particular of platelets post-burn injury. The more you know, the more you find you don’t know: the deeper you dive into the subject, the more you realise how incredibly complex the human body is. It seems somewhat presumptuous to think that with a little blob of PRP we can actually accelerate wound healing, which is a multifaceted orchestra at cellular level developed over several billions of years of evolution. However, should this overwhelming feeling of humility daunt us and prevent us from trying? No, we should proceed, in a systematic, careful and critical way, to unravel step-by-step the way to improve burn wound healing, ultimately leading to less or scarless healing.
REFERENCES


