Our experience in the treatment of chronic ulcers using Vivostat® PRF®. Series of 10 cases


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Abstract
The aim of this study is to demonstrate our clinical experience in the management of chronic ulcers of various aetiologies, through conservative treatment with autologous fibrin-rich gel plus platelet-derived growth factors, or PRP. This experience confirms the good results obtained in earlier studies relating to Plastic, Reconstructive and Aesthetic Surgery. Moreover, the point of difference with our study is the use of PRP as the only treatment for such lesions, without other adjuvant treatments, which is the usual approach in other works on this topic. From 2002 to 2007, we used PRP to treat ten patients with a history of conventional treatment failure, with an average age of 65.6 years. Three to five sessions per patient, with an interval between treatments of one week, were carried out. The method selected was the VIVOSTAT® PRF® System (MBA Group). Photographic monitoring was also performed during each session. We conducted visual tracking of the lesions (pre- and post-treatment), evaluating them according to a modified version of the scale designed by Valbonesi et al, which we referred to as the “Zaragoza Scale”. The outcome was poor in one patient, fair in two, good in five and excellent in two. Use of PRP for chronic ulcers in patients refractory to other treatments, both conservative and surgical, is now a real option in achieving a clear improvement and even a complete cure of lesions of this type.

Key words Chronic ulcers, Platelet-derived growth factors, Non-invasive treatment

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Introduction

The use of PRP, or platelet-rich plasma, as a sealing and hemostatic agent is a universally known and accepted therapeutic technique. Various studies and works (1, 2) demonstrate very convincing results over the past ten years and this technique has been used with great success in almost all branches of plastic surgery (oral surgery, hand surgery, aesthetic surgery) (3-5). However, the same scientific consensus has not been achieved with respect to the use of autologous platelet-derived growth factors alone in the treatment of chronic ulcers (6, 7). Various theories exist to explain this fact. Firstly, there is no unanimous position within the pharmaceutical industry as to how PRP should be obtained. We currently have various automatic systems for extraction of factors (8), but each one of these reflects different percentages of platelet-derived growth factors, both in vitro and in vivo. Secondly, there are also manual methods that can be used to obtain these factors, methods that are certainly less costly, but that do not guarantee a constant proportion of PRP because they are technician-dependent and, furthermore, they provide between 30% and 40% less PRP than automatic systems (8). Both questions greatly impede the search for consensus in the treatment of ulcers, wounds and hematomas, which could benefit from the anti-inflammatory, regenerative and hemostatic action of autologous PRP. This search for consensus has been further hindered by the rapid development, over the past few years in the USA, of recombinant human platelet-derived growth factors (9, 10), which have shown promising results. All of these factors lead to one question: do we continue to use autologous factors (more invasive and uncomfortable for the patient) or should we instead use recombinant factors? It is clear that we still need more ambitious prospective studies to resolve this dilemma.

Approximately two million people suffer from chronic ulcers caused by diabetes in the USA (11). The annual cost for care of these patients varies between 1.5 and 2 billion dollars, taking into consideration treatments, hospital admissions and medical consultations. In Spain, and more specifically in our hospital, the Department of Rehabilitation and Medullary Injury Patients performed a study (on seven patients admitted to that unit over 2001 and 2002) to determine the real cost of treatment of those patients who were suffering from progressively deteriorating pressure ulcers, despite having been given current front-line treatment (antibiotic creams, debriding substances, skin and flap grafts). The study observed that the cost to the public purse for management of these lesions was exorbitant (the number of unnecessary hospitalisations was 997 days with a real cost of €289,334), and that this did not resolve the issue of treatment of these patients and increased corresponding discomfort. On the basis of the very good results obtained in various studies (12-14) on the use of PRP in promoting healing of chronic ulcers, we decided to use the VIVOSTAT® PRF® (platelet-rich fibrin) system from the MBA Group (15) for extraction and application of platelet-derived growth factors + autologous fibrin in these lesions, so as to attempt to find a treatment for our patients’ condition.

Material and methods

Our study focused on ten patients over the period from 2002 to 2007. The mean age of the patients was 65.6 years. Of these patients, two were female and eight male. The etiology for the ulcerous lesions was vascular in six patients, pressure-based in three patients, post-surgical in one and post-traumatic in another. The ulcers were located on the lower limbs in six patients (Case 1: right internal malleolar region, Case 2: left pretibial region, Case 3: right internal supramalleolar region, Case 4: left internal malleolar region, Case 5: left pretibial region, Case 10: right pretibial region), in the sacral region in three patients and in the shoulder in one patient (Case 6: medial dorsal region) (Table I).

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Risk factors</th>
<th>Aetiology</th>
<th>Location</th>
<th>Sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>Female</td>
<td>Diabetes + Venous insuff.</td>
<td>Vascular</td>
<td>Lower limbs</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>Male</td>
<td>Diabetes + Venous insuff.</td>
<td>Vascular</td>
<td>Lower limbs</td>
<td>3</td>
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<tr>
<td>3</td>
<td>82</td>
<td>Male</td>
<td>Venous insuff.</td>
<td>Vascular</td>
<td>Lower limbs</td>
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<td>4</td>
<td>70</td>
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<td>Venous insuff.</td>
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<td>5</td>
<td>60</td>
<td>Male</td>
<td>Venous insuff.</td>
<td>Vascular</td>
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<td>6</td>
<td>57</td>
<td>Male</td>
<td>Colon cancer</td>
<td>Post-surgical</td>
<td>Shoulder</td>
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<td>Male</td>
<td>Paraplegia</td>
<td>Pressure</td>
<td>Sacrum</td>
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<td>35</td>
<td>Male</td>
<td>Paraplegia</td>
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<td>Sacrum</td>
<td>3</td>
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<tr>
<td>10</td>
<td>68</td>
<td>Female</td>
<td>Renal insuff.</td>
<td>Post-traumatic</td>
<td>Lower limbs</td>
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</table>
All patients were refractory to the usual conservative treatments used in clinical practice, involving both enzymatic and antibiotic debriding substances, and the various hydrocolloid dressings used. Furthermore, their severe concomitant conditions, such as type-2 diabetes (Cases 1, 2 and 6), both arterial and venous vascular insufficiency (Cases 1, 2, 3, 4, 5 and 10), renal insufficiency (Case 10), cancer (of the colon with bone metastasis in Case 6), paraplegia and advanced age, meant that surgery was inadvisable (partial skin grafts, local flap grafts or microsurgery).

To obtain autologous platelet-derived growth factors combined with fibrin, we selected the automatic VIVOSTAT® PRF® system from the MBA Group (15). Treatment took place on an outpatient basis without the need for hospital admission. The methodology for the process was as follows:

1. During each session, 120 ml of blood was extracted per patient and collected in a sterile disposable container (Figure 1), to which was added a phial of citrate (17.9 ml) as an anticoagulant and tranexamic acid as an antifibrinolytic. This was processed using double-centrifuging (Figure 2) to obtain 5 ml of plasma rich in growth factors and autologous fibrin (concentration 20 mg/ml). This process took approximately 23 minutes.

2. Samples were then taken for culture of the wounds and the ulcers were disinfected using saline and chlorhexidine washing.

3. Photographs were taken to allow monitoring of the course of treatment.

4. The platelet- and autologous fibrin-rich gel, buffered with two compounds (one bicarbonate and the other acetate plus calcium) to achieve a neutral pH, was then applied; the product was applied using a spraypen (Figures 3 and 4), a minimum distance of 5 cm from the problem area, until that area was well covered.
5. Final dressing with antibiotic vaseline gauze, covered with a suitable antiperspirant.
6. The dressing was not removed until the following session.

Results
To evaluate the progression of the ulcers and examine the regenerative power of the PRP, and in the absence of any surface analysis method as recommended by Montón et al (16), we developed a scoring scale by modifying the existing scale developed by Valbonesi et al (12) referring to the use of PRP in the treatment of chronic ulcers as an adjuvant to skin grafts, and based on subjective visual monitoring of the lesions, which we called the “Zaragoza Scale”. Scores allocated were 0 (no improvement) to 4 (complete healing) (Table II).

The results were analysed by a single surgeon, subjectively and on the basis of the visual scale described, one week following application of the final treatment session for each patient. Of the ten patients studied, in four the result was excellent (4), in three it was good (3), in two it was fair (2) and in one it was very poor (0) (Figures 5-13).

In cases 1, 2, 3, 4, 5 and 10, the first samples taken were positive for Methicillin-Resistant Staphylococcus Aureus (MRSA). Following completion of treatment with PRP, the cultures were negative for Case 5 (after five sessions) and Case 10 (after four sessions). In the remainder of these cases (6, 7, 8 and 9), the samples were negative both before commencement of treatment and after completion of the sessions (following four sessions in Case 6 and following three sessions in Cases 7, 8 and 9). However, the treatment results were poor in Case 1 (positive for infection pre- and post-treatment), fair in Cases 2 and 3 (positive for infection pre- and post-treatment), good in Cases 4 (positive for infection pre- and post-treatment), 5 (positive for infection pre-treatment and negative post-treatment), 6, 7 and 8 (negative for infection pre- and post-treatment), and excellent in Cases 9 (negative for infection pre- and post-treatment) and 10 (positive for infection pre-treatment and negative post-treatment) (Table III).

Discussion
The use of PRP in therapeutic processes related to Plastic, Reconstructive and Aesthetic Surgery is currently a real option in most public and private...
hospitals in our country; a situation that shows the advances that have occurred over recent years in tissue regeneration therapy. We are focusing here on the area of regenerative tissue treatment, but we should not forget the enormous potential that PRP could have as a haemostatic, anti-inflammatory and analgesic substance, effects that have been described extensively by other authors (6, 7).

The excellent results shown in recent articles (6, 16), although not equivalent in terms of extraction system for growth factors and autologous fibrin, number of patients and conditions to be treated, have only served to confirm the good outcomes described in our study. In seven of the ten patients that we treated (70%), the scores obtained following completion of the applications of PRP were good or excellent. In their study on 151 patients, Montón et al (16) show a similar degree of effectiveness in the treatment of chronic wounds of various aetiologies (predominantly vascular ulcers) when PRP alone was applied in 16 patients without other adjuvant treatments. Similarly, Eppley et al (6) performed a meta-analysis on three studies in which chronic ulcers were treated with PRP for eight to ten weeks, recording complete epithelisation of wounds in 81%, 93% and 100% of patients respectively.

The bactericide properties attributed to PRP in other studies were not clearly demonstrated in our work, as in only two cases out of six who commenced treatment with MRSA infection had the cultures become negative on completion of treatment. Paradoxically, however, the fact of being infected by MRSA on commencement of treatment was not a conclusive factor in obtaining a poor result (0-1), since three patients of the six infected achieved a score of good (3) or excellent (4). We can therefore say that although in our study PRP was not in most cases successful with MRSA, it did in fact clearly contribute to the healing of the wounds in 50% of patients who were infected.

Contrary to the results for other studies, such as the study by Eppley et al (6), in which optimal results were obtained with a greater number of applications (from eight to ten sessions), our study was more in agreement with the works by Montón et al (16) and Valbonesi et al (12), in which a greater number of sessions did not necessarily imply a better result, since in many
patients, one application was sufficient to produce evident improvement. In our series, three sessions were sufficient in the majority of patients to obtain a good result (score of 3 or 4). It is also true that, if we combine the parameters for infection, number of sessions and score, we can see that all of the patients who were given five sessions (three patients) were infected on commencement of treatment, a factor that could have had a negative impact on their final results.

With respect to the process for obtaining the PRP and fibrin, VIVOSTAT® provides a number of advantages and disadvantages compared with other automatic and manual systems for obtaining PRP (8, 18). Firstly, the fact that it is an automatic system facilitates its application by different operators, while that automation ensures that the end-product (PRP + autologous fibrin) will be the same in each patient irrespective of the session and the person applying it. We believe this to be an advantage compared to manual systems, which are highly operator-dependent. Another point we would like to emphasise is that the system is closed and sterile, which avoids the need for additional manipulation of the product before application of the PRP and thus possibilities for microbial contamination. Furthermore, the concentration of both PRP and fibrin achieved is nine times the baseline plasmatic concentration, a value considerably higher than for other automatic systems (8). It could perhaps be thought that the principal disadvantage of automatic systems is their high cost, but this argument cannot be sustained when we look at data from cost-benefit studies such as that conducted in our hospital by the Department of Rehabilitation and Medullary Injury Patients, which concluded that thousands of euros in unnecessary hospital admissions could be saved if treatment for chronic decubitus ulcers was provided using PRP in an outpatient context.

Conclusions

The fact that this was a pure observational study, i.e. subjective and limited by definition and, moreover, a study with a small study population, does not give us much basis for formulating conclusions. We should however highlight the fact that the good results obtained in our study should not necessarily be taken to mean that platelet-derived growth factors should replace conventional treatments (antibiotic creams, skin or flap grafts) as a front-line therapeutic option in the treatment of chronic ulcers, but rather that these factors may be of great value where these conventional treatments are unsuccessful or when the patient refuses surgical options.

In conclusion, the VIVOSTAT® PRF® system was shown to be of clinical and therapeutic value in the management of wounds in our patients. In the future, it would be very interesting to conduct a prospective study in a larger number of patients, to statistically validate the use of PRP in the treatment of chronic ulcers.


Comments on the work “Our experience in the treatment of chronic ulcers using Vivostat® PRF®. Series of 10 cases”

Dr Ithamar N. Stocchero
Co-ordinator of Plastic Surgery of the Hospital Santa Catarina and of the Centro Médico Viver Melhor, São Paulo, SP, Brazil.
Chairman of the Brazilian Association of Tissue Engineering and Stem-Cell Research

I should like to thank the authors for their interest in seeking a treatment for a group of persons who suffer and who are deprived of their normal social interactions and leisure activities, frequently at an age when they should be able to live with dignity with their families and friends, and who are unfortunately marginalised and humiliated by their
dependency in terms of care, treatment, transport and cost. This aspect of medical care within the plastic surgery discipline is often hidden and, therefore, impetus for corresponding treatment advances is lacking.

The authors themselves recognise the difficulty inherent in identifying the regenerative action of the solution they have proposed, PRP. Where and how does this substance act? According to the description of the cases presented, PRP has an action that is not related to the presence or absence of infection; despite the ischaemising factors, tissular hypoxia and advanced age of the patients, good results were obtained. Why? We still do not know. What is being demonstrated increasingly is that the human organism has within it the healing response for all of its ills. We simply need to learn to read the signs.

In an interesting analysis on factors that interfere with cicatrisation, Mustoe suggests that the synergy of factors is more important than the same factors in isolation. Rigotti (1) has been achieving brilliant results by infiltrating stromal vascular factors into areas of radionecrosis, and Yoshimura (2, 3) has been successful in creating a mixture that is effective in areas without cicatricial abnormalities. All of these facts merely demonstrate that we are still far from understanding the inflammatory and anti-inflammatory action taking place at the most fundamental levels of our systems. The proteins involved in the actions inducing inflammation, the principal driver for any regeneration, need to be better understood, because they can lead to a good or bad outcome. Why do they stop producing a beneficial action?

We (4) are studying these actions in parallel investigations, seeking a common thread in all of these factors (PRP, SVF, CAL, GF etc), because it is not a combination of these factors that heals, but rather an element present in them that has not yet been determined. We know that it exists but not what it is.

I would like once again to thank the authors for the promising results they have achieved and for their desire to seek a solution to these issues using a
treatment proposal that is balanced, repeatable, confirmed and supported. This proposal will eliminate technician-dependent errors, providing a model to be used as a reference. It suggests a methodology to be applied in an area where comparison is difficult because of the quantity of variables present. Although we must agree that this proposal does not identify the action responsible for regeneration, ultimately it is more important to heal than to understand what will heal; in time, we will be able to identify the true nature of that healing.

Response to the comments from Dr Ithamar N. Stocchero

Dr Enrique Monclús

Firstly, I would like to thank Professor Stocchero for his words of support. When a Chairman of the Brazilian Association of Tissue Engineering recognises that you are on the right track, it is good reason to be happy and proud of the work you have done.

With reference to this issue, as the Professor stated in his comments, although we now understand many of the molecules involved in the inflammatory cascade (PDGF (platelet-derived growth factor), TGF (transforming growth factor), Interleukin-1, VEGF (vascular endothelial growth factor), EGF (epidermal growth factor), IGF (insulin-like growth factor), etc), there are many others that we do not yet know and understand. It is true that in their magnificent article, the current reference on the use of PRP in plastic surgery worldwide, Eppley et al provided a detailed list of all of the physiological effects of platelet-derived growth

Bibliography


factors, including tissue regeneration and haemostatic effects. They also noted the antibacterial effect attributed by certain studies (Klinger et al), although our study unfortunately did not, as we explained in the discussion of our study, provide any proof of that effect. We do intend in the future to investigate the reasons for this.

To finish, I would simply like to say that I am sure that very soon, as has been suggested by our Brazilian colleagues in their study published in this journal (Almeida et al), the incorporation of the use of adipose-derived stem-cells combined with the various growth factors listed above will contribute significantly to the field of reconstructive surgery.