Use of platelet rich plasma and hyaluronic acid on exposed tendons of the foot and ankle

Platelet rich plasma was used as an autologous scaffold for cellular growth, in combination with hyaluronic acid as a temporary dermal substitute. This aided healing of acute and chronic open wounds of the foot and ankle.

Platelet rich plasma; hyaluronic acid; complex wound; tendon exposure

Traumatic injuries of the foot and ankle are often associated with significant skin loss, resulting in the exposure of tendons, bone, or implants. These injuries are, in many ways, similar to the chronic foot ulcers associated with ischaemic diseases, such as diabetes mellitus. Rapid formation of granulation tissue and blood vessels is essential for the healing of these complex wounds.

Traditionally, wound management involves frequent wet dressing changes (three to four times daily), but in our experience this treatment can be protracted and painful. Furthermore, through the activity of its collagenase and metalloproteinase constituents, interstitial fluid from open wounds reduces the local blood supply and disturbs wound healing.

Soft tissue defects of the foot and leg usually require local or free flap surgery if granulation tissue formation is limited and a skin graft procedure is not applicable. A split-thickness skin graft is not recommended for wounds with exposed bone or neurovascular structures, or for wounds involving the weight-bearing surface of the foot.

Tissue-engineered tendons are a potentially significant source of readily available tissue for use in both tendon and ligament reconstruction. Current tissue-engineering strategies predominantly rely on scaffolds that consist of synthetic (polyglycolic acid) and naturally derived material (collagen) to form a cell-scaffold construct.

The development of therapeutic, autologous formulations that control the levels of growth factors and their local release at wound sites is instrumental in achieving a successful outcome. Potential examples are platelet rich plasma (PRP), which can be used as an autologous scaffold for cellular growth, and hyaluronic acid (HA), which can act as a temporary dermal substitute.

Platelet rich plasma

During normal tissue repair in vivo, platelets release high concentrations of biologically active proteins including growth factors. In doing so, they are able to influence a wide range of processes and promote the recruitment, growth and morphogenesis of cells. The ability of platelets to release growth factors and cytokines at supra-physiological concentrations within a growing clot can be harnessed therapeutically to accelerate natural healing.

PRP is developed from autologous blood and consists of a volume of autologous plasma with a platelet concentration above the baseline. When PRP is combined with autologous thrombin and/or batroxobin (gelation-inducing enzyme) and/or calcium chloride, platelet gel is created. This gel is a rich source of growth factors.

Platelet activation induces the release of several early (bFGF, PDGF and IGF) and delayed growth factors (EGF, VEGF, TGF-b, IGF).

The liquid PRP and platelet gel are obtained after centrifugation of 10ml of venous blood. Platelet gel is extremely malleable and so can be used in a variety of clinical scenarios, including bone grafts and ulcers. Once prepared, the PRP can be stored for any future applications.

Autologous PRP has been safely used in many surgical fields, including:
- Orthopaedic
- Maxillofacial
- Cardiothoracic
- Plastics and reconstruction.

It has been used in the treatment of non-healing wounds, including trophic and vascular ulcers, pressure ulcers, fistulae, burns and demodermepidermal dystrophies.

PRP has been used as an autologous scaffold for cellular growth, and it has been proved that, in association with mesenchymal stem cells, it potentiates tissue regeneration.
Table 1. The open wound scoring system

<table>
<thead>
<tr>
<th>Grade</th>
<th>Wound status</th>
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<tbody>
<tr>
<td>0</td>
<td>Closed wound</td>
</tr>
<tr>
<td>1</td>
<td>Skin or soft tissue defect</td>
</tr>
<tr>
<td>2</td>
<td>Bone, tendon, implant exposure (any one)</td>
</tr>
<tr>
<td>3</td>
<td>Bone, tendon, implant exposure</td>
</tr>
<tr>
<td></td>
<td>(any combination of two or more)</td>
</tr>
<tr>
<td>4</td>
<td>Associated or residual infection</td>
</tr>
</tbody>
</table>

**Hyaluronic acid**

HA is a major component of the extracellular matrix (ECM). It is a non-sulphated, linear glycosaminoglycan consisting of repeated units of glucuronic acid and N-acetyl-glucosamine. Its physiological functions relate to its structural role within the ECM and its ability to interact with cell surface receptors. It is able to hydrate and modulate the cellular microenvironment, while its cell surface receptor bindings induce cell-cell adhesions, cell-substrate adhesions, proliferations, and cell migrations. In this way, HA not only facilitates the entry of a large number of cells to the wound site but also contributes to the orientation of the fibrous component of the ECM.12,13

HA can act as a temporary dermal substitute in the treatment of wounds. It forms a three-dimensional scaffold that is promptly colonised by fibroblasts and ECM components, favouring the ordered reconstruction of dermal tissue.14,15

HA acts as a scaffold for the PRP. An ideal scaffold should promote rapid remodelling, possess increased strength, demonstrate improved healing and permit early rehabilitation and return to function after implantation. In addition, the availability of such a scaffold would reduce the likelihood of donor site morbidity, increased surgical time, increased cost and poor function associated with autologous tendon harvest (currently considered the ‘gold standard’ in clinical practice).

In summary, an optimised scaffold would have the potential to vastly improve the treatment of tendon and ligament injuries, especially those associated with tumour, trauma, and congenital deficiencies where autograft or allograft tissue might not be available in sufficient quantity for reconstruction.3

**Objectives**

We propose that PRP and HA can be used to cover exposed bone or soft tissue defects without the need for frequent dressing changes, and their use reduces chronic oedema and increases local blood supply, which enhances the formation of healthy granulation tissue.

New reports have been issued on the application of PRP to soft tissue defects of the extremities, abdomen, chest and face.16 However, reports regarding its use in foot and ankle wounds are limited.

The purpose of this retrospective evaluation was to determine how HA and PRP help wound healing and whether or not their use can reduce the need for flap surgery in the treatment of acute or chronic open wounds in the foot and ankle region.

**Materials and method**

All 30 patients with tendon exposure and loss of substance of the foot/ankle treated at the Department of Plastic and Reconstructive Surgery at the University of Rome Tor Vergata in the previous two years were included in this retrospective study.

Inclusion criteria were:
- Patients aged 20–80 years
- Chronic injury
- Burns or traumatic orthopaedic aetiology
- Use of advanced dressings for at least two weeks.

Exclusion criteria were:
- Cancer
- Autoimmune diseases
- Haematological diseases
- Chemotherapy or radiotherapy
- Treatment with antiplatelet agents
- Septicaemia.

All patients were enrolled following a cycle of advanced dressing, which allowed preparation of the wound bed. Intraoperative surgical debridement was performed.

Patient anonymity was respected and written informed consent was obtained before the surgical procedure and the production of images. This retrospective evaluation was approved by the research ethics board of our institution.

The intervention

A self-contained disposable kit (Regen Lab, Mollens, Switzerland) was used to process 16ml of peripheral venous blood. The kit consisted of two or more sterile, evacuated blood-collection tubes, needles, and a transfer device. Blood was collected in two Regen thrombocyte harvesting tubes (THT) (8ml each) and autologous thrombin was obtained by treating 7ml of patient blood with a Regen autologous thrombin serum tube. All tubes were centrifuged at 1500g (corresponding to 3000rpm) for 12 minutes in a Hettich Rotofix Centrifuge, and PRP was obtained.

The PRP obtained through this method was of a very high quality, with platelet and growth factor contents equal to the highest levels obtained in previously published studies.17–20

PRP may be injected intralesionally, intratendonally or perilesionally, or mixed with autologous thrombin in a 9:1 ratio forming platelet gel, which
may then be used topically (activated with calcium gluconate).

PRP gel was sutured to the tendons with 4/00 vycril (Fig 1-2). The wounds were covered with a three-dimensional polymerised HA-medicated biological dressing (Fig 3). HA can be left in place for 15–20 days when growth factor action peaks, therefore drug therapy can be reduced, which might benefit the patient. Where necessary, elasto-compression could be used to avoid venous-lymphatic interference with the regeneration process.

Assessment
To assess the status of wounds in terms of their openness, the following scoring system was used at 15, 21 and 30 days following intervention:

- 0 = closed wound
- 1 = skin or soft tissue defect
- 2 = bone, tendon, implant exposure (any one)
- 3 = bone, tendon, implant exposure (any combination of two or more)
- 4 = associated or residual infection.

Comparisons between the assessments performed at 15 and 21 days, and days 21 and 30 days were done using the Mann-Whitney U test.

Results
The results of the wound status assessments, shown in Fig 4, reveal median values of 3 (after 15 days), 1 (after 21 days) and 0 (after 30 days), with a progressive significant reduction in score from 15 to 21 days (p<0.001) and from 21 to 30 days (p<0.001).

Only one patient had a score of 0 at the first assessment (15 days following the intervention), seven had a score of 0 at the second assessment and 28 had a score of 0 at the third.

Only two patients had wounds that remained open after 30 days. One of these patients had scores of 4 at day 15 and 2 at days 21 and 30. The other patient had a score of 2 at days 15 and 21 and a score of 1 at day 30.

All other patients had a score of 0 at the third assessment, 30 days following the intervention.

One complication (skin graft scar contracture) was reported.
Discussion

The severity of open wounds was noticeably reduced after treatment with PRP and HA — only one patient needed a free flap to cover exposed bone and tendon. Prevention of deep infection is essential during the treatment of soft tissue defects, and simple wet dressing may not be adequate in this context as wounds are inevitably exposed to the atmosphere.

In this evaluation, no case of infection was recorded during the treatment period. Accordingly, we consider that PRP probably also reduces the infection rate in soft tissue defects. The one complication reported, skin graft scar contracture, can potentially limit foot function, although successful scar release was achieved in this case.

This evaluation has several limitations that require consideration, namely that we had a small data set and there was no control group, which severely limited objectivity of the evaluation.

We did not see the value in treating patients with either PRP or HA individually as previous studies have shown that, compared with their joint application, healing takes longer and the number of applications is increased.10,21

Although further research is needed, our results suggest that our technique is effective. We suggest that a prospective randomised multicenter trial be undertaken to determine the merits of PRP and HA for the treatment of soft tissue defects of the leg and foot. However, based on the results of previous studies of its use in the treatment of injuries at different locations, it appears that using PRP and HA together significantly enhances the formation of granulation tissue and helps prevent infection.22

Our results add to growing evidence that PRP and HA are useful adjunctive treatments for open wounds. This evaluation found that the rapid formation of granulation tissue was facilitated, healing times were shortened, and the need for additional soft tissue reconstructive surgery was reduced.

References