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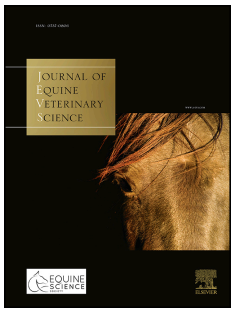

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Platelet-Rich Plasma combined with a sterile 3D polylactic acid scaffold for postoperative management of complete hoof wall resection for keratoma in four horses.

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Authors' declaration of interests

The authors declare no conflicts of interest.

Abstract

Keratoma is a non-malignant horse tumour that grows in the space between the horn of the hoof and the distal phalanx. Keratoma causes lameness in the horse, and surgical excision is the treatment of choice. Four horses underwent removal of a keratoma by complete hoof wall resection. The remaining wound was treated with Platelet-Rich Plasma (PRP) combined with a sterile 3D polylactic acid scaffold. The PRP was applied at 3, 6, 9, 12, 15 and 18 days postoperatively. The surgical site was cleaned with gauzes and swabs soaked in Ringer's lactate solution before applying PRP and the foot bandage. Healthy granulation tissue developed at 6-21 days postoperatively. The hoof wall defect was completely filled with new hoof wall within 6-8 months after surgery. All horses returned to their previous exercise level and no recurrence of lameness was reported by the owner.

*Keywords***:** Platelet-Rich Plasma; PRP; regenerative medicine therapy; keratoma; horse.

1. Introduction

Keratoma is a benign tumour that originates from the keratin-producing epidermal cells of the coronary band [1]. Intermittent lameness and chronic drainage at the white line are the most common clinical signs [2]. The only effective treatment for keratoma is complete surgical excision of the mass. Complete hoof resection, partial hoof resection, and supracoronary approach have been reported for keratoma removal [1,3]. Postoperative treatment of the remaining wound requires topical antibiotics (e.g., enrofloxacin and metronidazole power, amikacin impregnated collagen sponge), systemic antibiotics to prevent infection, nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce postoperative pain, special shoes to stabilise the hoof, bandages to protect the healing tissues (e.g., gauze soaked in betadine solution), and synthetic resin to fill the defect [1-4]. Period of convalescence is related to surgical techniques, proximal extent of the abnormal tissue, size of the window made in the hoof wall, location of the defect around the hoof, and postoperative

management [1,5]. Based on the literature, the time required for the regrowth of the hoof wall is usually of several months, and horses may return to their previous exercise level after 6-36 months postoperatively [1].

Platelets are a natural source of growth factors and cytokines that induce wound healing. Platelet-Rich Plasma (PRP) is an autologous blood-derived biomaterial, safe and biocompatible whose activity in regenerative medicine has been widely demonstrated in animals [6-8]. It is commonly used for treatment of skin wounds [9], and tendon and ligament injuries in horses [10].

The present work describes for the first time the feasibility of the use of PRP for postoperative management of the remaining wound in the hoof wall, after its complete resection for keratoma in four horses, with the aim of reducing both medical treatment and recovery time.

2. Case Details

2.1 History and Clinical Findings

Four horses were referred for lameness.

Case 1. An 8-year-old Argentine Criollo gelding was referred with a 12-month history of draining tracts from the supracoronary band region and subsolar region of the left hind hoof. Treatment with NSAIDs and antibiotics had not been successful. The horse was 3/5 lame. Draining tracts were detected at supracoronary band and subsolar region of the left hind hoof. Hoof testers revealed an area of sensitivity over the dorsomedial region of the toe.

Case 2. A 10-year-old Friesian gelding was referred with a 12-month history of draining tracts from the subsolar region of the right hind hoof. The horse was 3/5 lame. Examination of the right hind foot with hoof testers revealed an area of marked tenderness over the dorsomedial region of the toe. Mucopurulent fluid was identified coming from draining tracts in the subsolar region.

Case 3. A 17-year-old Friesian mare was referred with an 8-month history of cutaneous draining tracts from the supracoronary band region of the right front hoof. Only a temporary relief of the lesion was obtained with antibiotics and NSAIDs. The horse was 3/5 lame. There was a vertical

fissure originated at level of the proximal right front hoof, extended distally and completely through the hoof to the underlying tissues. A necrotic area was identified in the subsolar region.

Case 4. A 9-year-old Quarter Horse gelding was referred with a 3-month history of subsolar abscess of the left front hoof. Surgical treatment combined with antibiotics and NSAIDs had not been successful. The horse was 4/5 lame. Examination of the left front foot revealed a bulge of the dorsomedial aspect of the hoof. Hoof testers revealed an area of sensitivity over the dorsomedial region of the toe.

2.2 Diagnosis and Surgery

Diagnosis of keratoma was based on clinical history, clinical findings, and radiographic findings. Radiographs of the foot revealed a radiolucent area in the solar margin of the distal phalanx in all horses (Fig. 1).

The keratoma was removed via complete hoof wall resection in all horses [1]. Before surgery, 25 mm thickness wooden shoes were applied in a non-traumatic manner to both hoofs (front or hind) to prevent the occurrence of laminitis as well as hoof wall instability caused by the removal of a wide section of hoof wall [11]. A recess was created in the wooden shoe by cutting a half-moon shaped corresponding to the portion of the hoof wall to be surgically removed (Fig. 2). All surgical procedures were performed in the standing sedated horse. Animals received 10 µg/kg of detomidine (Domosedan, Orion Pharma, Milan, Italy) combined with 10 µg/kg of butorphanol (Nargesic, Acme, Reggio Emilia, Italy) intravenously [12]. Medial and lateral palmar/plantar nerve blocks were performed by injecting 3 mL 0.5% bupivacaine hydrochloride (Bupivacaina Ang., Angelini, Rome, Italy) [1]. The excised mass was submitted for histopathology which confirmed the diagnosis of keratoma in all horses. The absence of bacterial contamination was ascertained by submitting swab collected from the surgical site to a microbiological test.

2.3 PRP Preparation

A standardized technique of double centrifugation was used to prepare PRP [7]. Three blood collections were performed from each horse. A blood bag (Teruflex, Terumo Italy, Rome, Italy) with citrate phosphate dextrose adenine (CPDA-1) was used for the collection of 250 mL ± 10% whole blood. The blood was aliquoted into five sterile 50 mL Falcon centrifuge tubes. The tubes were centrifuged at 180xg/20min in a swinging rotor, promoting the separation of the plasma containing the platelets from buffy coat and red blood cells pellet. The plasma was drawn by sterile plastic transfer pipette to another 50mL Falcon centrifuge tube and was centrifuged again at 900xg/15 min. The platelet pellet was resuspended in a small volume of plasma (5 mL) and cells counted by a haematology analyser (Cell-Dyn 3500R, Abbott, Chicago, USA). Finally, PPP was added to PRP in order to obtain a platelet concentration of 10^9/mL [7]. A microbiological test of the PRP preparation was first performed to assess its sterility. To this aim an aliquot of PRP was layered on the surface of Blood Agar plate, and the absence of colonies was evaluated after an overnight incubation at 37°C. Afterwards, PRP was activated with 10% calcium gluconate to promote PRP adhesion to the surgical site. If PRP was not immediately used, it was freezed at -80°C and stored until the application, and a platelet lysate (PL) was obtained. Then, approximately 15 mL of PRP (or PL) were gelled by activation with 10% calcium gluconate. The gels were prepared in sterile Petri dishes containing a sterile 3D polylactic acid (PLA) scaffold (Prometheus, Parma, Italy) which was produced through 3D printing using an FDM 3D printer [10,13] (Figs 3A-3C). A medical grade pure PLA reel (Verbatim PLA) was used. Shape, geometry and porosity of scaffolds were controlled creating a CAD file, converting it in a G-CODE through which the FDM printer is able to produce the designed scaffold. Scaffolds had thicknesses ranging between 100 and 500 µm. Shapes and dimensions were adjusted depending on the specific clinical case and according to shape and dimension of treated wounds. The geometry was characterized by a reticular structure and pores had variable dimensions, ranging between 0.5 and 1 mm. The gel adhering to the scaffold was shaped to cover precisely the surgical site immediately before its application (Figs. 3D and 3E).

2.4 Postoperative Care and PRP Clinical Use

All horses were hospitalized and were given phenylbutazone paste (Bute, Acme, Reggio Emilia, Italy) 1 g orally once a day for 7 days postoperatively. A foot bandage was applied and changed every 24 h for 3 days. Surgical site was first evaluated for occurrence of signs of infection (e.g., the presence of purulent discharge) [14] and then cleaned with gauzes and swabs soaked in Ringer's lactate solution before reapplying the foot bandage. PRP gel combined with a sterile 3D PLA scaffold was applied at the surgical site at 3, 6, 9, 12, 15 and 18 days postoperatively. The surgical site was cleaned as previously described before applying the PRP. The foot bandage was reapplied after PRP application. Every foot bandage was performed as follows: sterile gauzes soaked with Ringer's lactate solution were firstly applied either to the exposed laminae, or over the PRP gel combined with a sterile 3D PLA scaffold; then, the foot was bandaged with sterile combine roll, gauze roll and elastic tape.

2.5 Outcome

A healthy granulation tissue developed within the third PRP application in all horses except in case number 4 (Fig. 4). No postoperative complications (e.g., infection, exuberant granulation tissue, recurrent growth of keratoma) were recorded, and horses were hospitalized for 24-30 days. The wooden shoes were removed, and aluminium horseshoes (Fig. 5) were applied before patient's discharge. The owner was given postoperative instructions to rest his horse for approximately one month and to change the foot bandage every 3 days until the new hoof wall had grown. The hoof wall defect was completely filled with new hoof wall within eight months after surgery (Fig. 6). All horses returned to their previous exercise level and no recurrence of lameness was reported by the owner (Table 1).

3. Discussion

Although the number of treated animals is limited, the use of PRP seems to reduce both the time required for the regrowth of the hoof wall and the period of convalescence in our patients compared to previous studies [1-4]. In our series, the hoof wall took 6-8 months to grow out, and all horses returned to their previous level of performance at 8-12 months postoperatively. It is likely that PRP may have contributed to establishing an anti-inflammatory and pro-angiogenic environment, and may have stimulated the injured tissue to restore its functional integrity [8]. In fact, it is well demonstrated that PRP exhibits anti-inflammatory properties, exerts anti-oxidative effects protecting against hypoxia/reperfusion injury, preserve the endothelial integrity, and promote angiogenesis [9]. Furthermore, it is likely that the 3D PLA scaffold provided a physical framework suitable for cell adhesion and migration into the defect for healing to progress [10]. As a matter of fact, the PLA scaffold provided a suitable support to handle PRP gels, allowing its precise positioning to cover the wound.

No topical iodine solutions were applied because these substances may affect platelet vitality [7]. We preferred to use Ringer's lactate solution, which contains a high concentration of calcium ions promoting platelets adhesion to the surgical site [15]. Even though postoperative administration of antibiotics is recommended, no topical and systemic antibiotics were administered because no signs of infection were detected. The absence of infection is likely related to the frequent cleaning of the surgical site and replacement of the hoof bandage. Nevertheless, PRP may also have contributed to the maintenance of sterility because it can exhibit immunomodulatory and anti-bacterial properties. In fact, PRP is a concentrate of growth factors which induce chemotaxis of neutrophil and monocyte in the wound site [8]. Even though further studies are needed, the use of PRP for management of many pathological conditions might reduce the administration of antibiotics to animals and, consequently, could help to decrease antimicrobial resistance.

In conclusion, although we are aware that we cannot attribute with certainty the improved post-surgical outcome of horse keratoma reported here exclusively to the local application of PRP, growth factors released by platelet degranulation may have affected fibroblast recruitment and

proliferation, matrix remodelling [8] promoting proper healing of the remaining wound in the hoof wall reducing the recovery period.

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Animal welfare/Ethical statement

The present report did not use any animals for the purpose of scientific discovery. This is a retrospective study and does not require ethical approval.

Authorship

F.L. and G.L.C. were responsible for conceptualization, and anesthetic management. M.A., C.B., B.B., and L.P. were responsible for methodology, diagnosis, surgical procedures, and postoperative management. V.C., S.G., and R.R. were responsible for methodology, PRP preparation and application. All authors contributed equally to the writing of the manuscript.

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226 **Table**

227 Table 1

228 Outcome and follow-up of the patients. Times are expressed as days, months, and years after

229 surgery.

Follow-up data	Case 1	Case 2	Case 3	Case 4
Development of healthy granulation tissue (days)	12	6	9	21
Discharge (days)	24	24	30	30
Light exercise (months)	2	2	2	2
Filling of the hoof defect with new hoof wall (months)	6	6	8	7
Return to previous exercise level (months)	8	10	12	12
Available follow-up (years)	3	1	2	2

230

Figures legend

Fig. 1. Area of bone lysis in the solar margin (arrow).

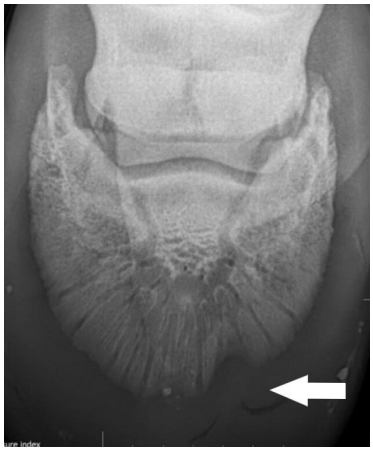
Fig. 2. Lateral (A) and plantar (B) views of wooden shoe applied to the hoof to be surgically treated.

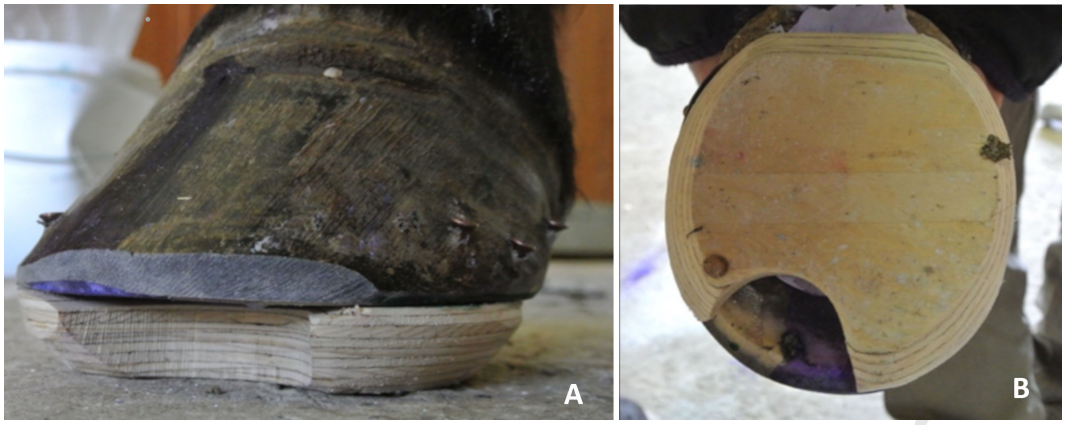
Fig. 3. Production process of sterile 3D polylactic acid scaffold combined with PRP gel (A). The combination between the 3D polylactic acid scaffold (B) and the platelet gel is prepared inside a Petri dish (C). The patch is taken from the dish (D) and cut to the correct size with a lancet just before its application (E).

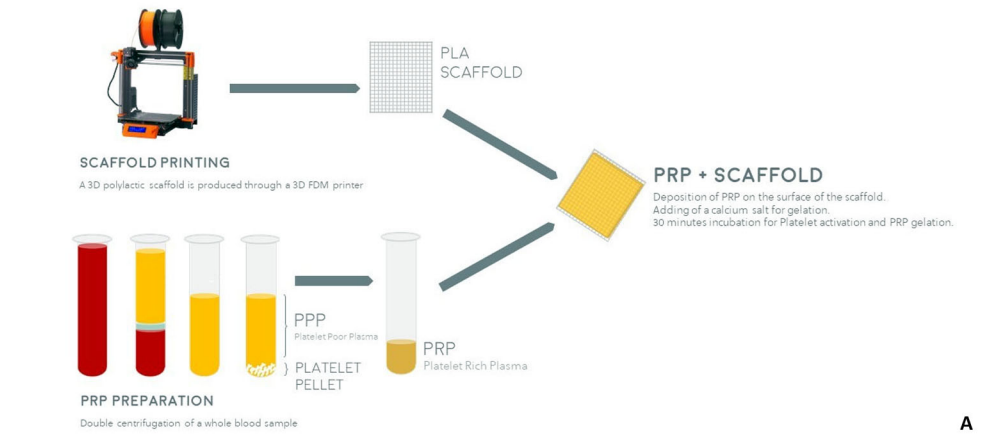
Fig. 4. A healthy granulation tissue developed after, respectively, the third PRP application in case 1 (A, surgical site at 12 days postoperatively), the first PRP application in case 2 (B, surgical site at 6 days postoperatively), the second PRP application in case 3 (C, surgical site at 9 days postoperatively), and the sixth PRP application in case 4 (D, surgical site at 21 days postoperatively).

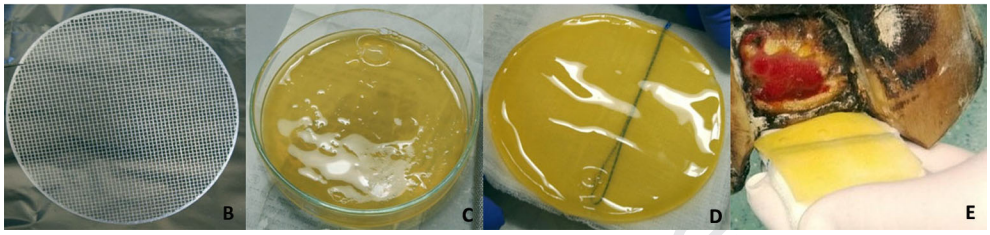
Fig. 5. Aluminium horseshoe. A blue plastic slab was placed between the hoof and the horseshoe to make the shoe more comfortable and to prevent damage from concussions.

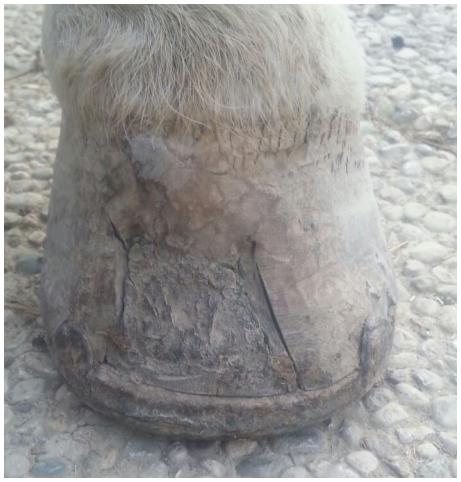
Fig. 6. Filling of the hoof defect with new hoof wall at 6 months postoperatively (case 1).











- Keratoma is a tumour that grows between horn of the foot and distal phalanx.
- Surgical excision is the treatment of choice.
- PRP was combined with a sterile 3D polylactic acid scaffold.
- PRP was applied to cover the remaining wound after complete hoof wall resection.
- PRP reduces both time required for regrowth of the hoof wall and the period of convalescence.

Ethical statement

The present report did not use any animals for the purpose of scientific discovery. This is a retrospective study and does not require ethical approval.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.