https://doi.org/10.52973/rcfcv-e35717 Revista Científica, FCV-LUZ / Vol. XXXV

Preparation and clinical application of thrombocyte-leukocyte rich plasma in green turtles (*Chelonia mydas*). Technical note

Preparación y aplicación clínica de plasma rico en trombocitos y leucocitos en la tortuga verde (*Chelonia mydas*). Nota técnica

***Agustina Algorta*[1], *Fernando Fumagalli*[2], *Virginia Ferrando*[3], *Victoria Sorriba*[4], *Adrián Carzoli*[4], *Kevin Yaneselli*[1*]**

[1]*Unidad de Inmunología e Inmunoterapia, Departamento de Patobiología, Facultad de Veterinaria, Universidad de la República (Udelar), PC:13000, Montevideo, Uruguay.*
[2]*Unidad de Clínica Semiológica, Departamento de Clínicas y Hospital Veterinario, Facultad de Veterinaria, Universidad de la República (Udelar), Montevideo, Uruguay.*
[3]*ONG Karumbé, Conservación de tortugas Marinas del Uruguay, Uruguay.*
[4]*Unidad de Imagenología, Análisis Clínicos y LEMA, Departamento de Clínicas y Hospital Veterinario, Facultad de Veterinaria, Universidad de la República (Udelar), Montevideo, Uruguay.*
**Autor correspondencia: kevin.yaneselli@fvet.edu.uy*

ABSTRACT

Traumatic injuries in turtles pose a significant challenge for veterinarians, requiring extended follow-up and rehabilitation periods. With conventional healing methods often yielding limited results, the realm of regenerative medicine in reptiles and amphibians has offered promising alternatives. Among these, the utilization of thrombocyte-leukocyte rich plasma and analogous hemocomponents akin to platelet-rich plasma in mammals has garnered attention. Hence, the objective of this study was to explore therapeutic hemocomponents in reptiles, specifically thrombocyte-leukocyte rich plasma, and its application in a clinical case involving *Chelonia mydas*. Autologous thrombocyte-leukocyte rich plasma was prepared via a density gradient centrifugation process to isolate the buffy coat and concentrate thrombocytes and leukocytes in the final formulation. The selected clinical case involved a green turtle (*Chelonia mydas*) undergoing rehabilitation at the Karumbé center, exhibiting lesions and abscesses in the jaw and flipper regions. Treatment entailed abscess removal, antibiotic administration, and the application of gelified thrombocyte-leukocyte rich plasma in the affected areas. Results demonstrated a recovery rate of 20.8% for thrombocytes and leukocytesin the thrombocyte-leukocyte rich plasma preparation, which effectively gelled upon application. Regarding the clinical case evolution, after 2 months of follow-up, an improvement in wound healing was observed. In conclusion, this is the first report of the preparation of thrombocyte-leukocyte rich plasma in *Chelonia mydas* and its application in a clinical case with favorable progression. However, more cases are needed in this species and type of lesion to obtain conclusive results on its beneficial effects in turtles.

Key words: Marine turtle; thrombocytes; blood plasma; wound; abscess

RESUMEN

Las lesiones traumáticas en tortugas pueden ser frustrantes para los veterinarios y requerir largos tiempos de seguimiento y rehabilitación. Ante la limitante de cicatrización han surgido novedosas opciones desde la medicina regenerativa en reptiles y anfibios como el uso de plasma rico en trombocitos y leucocitos y otros hemocomponentes comparables con el plasma rico en plaquetas en mamíferos. Por ello, el objetivo del presente trabajo fue desarrollar hemocomponentes terapéuticos en réptiles como el plasma rico en trombocitos y leucocitos y su aplicación en un caso clínico de tortuga verde (*Chelonia mydas)*. La preparación de plasma rico en trombocitos y leucocitos autólogo se preparó mediante un procedimiento de centrifugación en gradiente de densidad para la separación de la capa flogística y concentración de trombocitos y leucocitos en la formulación final. El caso clínico seleccionado fue una tortuga verde (*Chelonia mydas*) que se encontraba en rehabilitación en el centro Karumbé. Presentaba lesiones y abscesos en la zona de la mandíbula y en las aletas pectorales. El tratamiento consistió en la remoción de los abscesos y uso de antibióticos, a lo que se agregó depósito del plasma rico en trombocitos y leucocitos gelificado en las zonas tratadas. Los resultados mostraron que se pudo recuperar un 20,8 % de los trombocitos y leucocitos para el preparado de plasma rico en trombocitos y leucocitos y gelificar al momento de aplicación. En cuanto a la evolución del caso clínico, a los 2 meses de seguimiento, se observó una mejoría en la cicatrización de las heridas. En conclusión, fue posible por primera vez reportar la preparación de plasma rico en trombocitos y leucocitos en *Chelonia mydas* y su aplicación en un caso clínico que tuvo una evolución favorable. Asimismo, es necesaria mayor casuística en esta especie y tipo de lesión para obtener resultados concluyentes sobre su efecto beneficioso en tortugas.

Palabras clave: Tortuga marina; trombocitos; plasma sanguíneo; herida; absceso.

Recibido: 04/06/2025 Aceptado:19/08/2025 Publicado: 24/09/2025

INTRODUCTION

The green turtle (*Chelonia mydas*) is a marine species that feeds and develops in tropical and subtropical oceans, with adults predominantly residing in the South Atlantic Ocean [1 , 2 , 3]. It is listed as an endangered species on the International Union for Conservation of Nature (IUCN) Red List [4]. In Uruguay, the non-governmental organization Karumbé has operated a Sea Turtle Rehabilitation Program continuously since 2000 [5].

The capture, rehabilitation, and reintroduction of wild animals are common practices in marine wildlife rescue centers. Reducing the length of stay in these facilities is crucial to ensure animal welfare and optimize resources. However, traumatic injuries—caused by predation or fishing gear—are common in both captive and wild turtles. These injuries, affecting soft tissues or bone, often require prolonged recovery, monitoring, and treatment, delaying rehabilitation and complicating reintroduction into the wild [6 , 7 , 8].

Therefore, it is necessary to advance therapeutic methods that accelerate healing and shorten recovery periods. One emerging option is the use of hemocomponents to promote wound healing in turtles. This approach is based on studies in mammals demonstrating the benefits of platelet-rich plasma (PRP) and platelet-rich fibrin, both of which concentrate growth factors and cytokines to enhance tissue regeneration. These blood derivatives have gained popularity in both human and veterinary medicine as cost-effective and safe therapeutic options [9 , 10 , 11].

In turtles, the application of thrombocyte-leukocyte rich plasma (TLRP) was first reported in 2015 for treating skin and bone injuries in *Testudo* spp., showing favorable clinical outcomes [12]. Similarly, Chen and Deem [7] reported encouraging results using hemocomponents to treat a nasal tip wound in a terrestrial tortoise (*Indotestudo elongata*) after conventional antibiotic therapy and debridement failed.

In reptiles, TLRP has also been used to treat chronic oral cavity disorders in a python and a chameleon, with evidence suggesting potential regenerative benefits [10]. However, therapeutic use of hemocomponents in reptiles and amphibians remains limited, though initial findings support further exploration in herpetological medicine. Thus, the objective of this study was to develop therapeutic hemocomponents, specifically TLRP, in turtles sea and evaluate their clinical application in a case involving a green turtle (*Chelonia mydas*).

MATERIALS AND METHODS

TLRP preparation

Autologous TLRP was prepared following the density-gradient centrifugation protocol described by Di Ianni *et al.* [12]. Briefly, 13 mL of blood were drawn from the external jugular vein (cervical venous sinus) using a sterile syringe preloaded with 3.8% sodium citrate as an anticoagulant.

The sample was centrifuged at 250 *g* (Thermo IEL CL30R centrifuge, USA) for 15 min to separate the plasma, and the cellular fraction was resuspended in an equal volume of phosphate-buffered saline (PBS). This diluted fraction was layered onto a 1:1 lymphocyte separation medium (density: 1077 g/mL) and centrifuged at 150 *g* for 20 min. The thrombocyte-leukocyte (T-L) fraction was collected and washed with PBS. Finally, T-L cells were resuspended in 20–25% of the original plasma volume (FIG. 1).

T-L counts were performed using a Neubauer chamber (Marienfeld, Neubauer bright-line, Germany) on both whole blood and TLRP samples. The recovery percentage of T-L was calculated using the formula: Cell recovery (%) = (100 × T-Lr) / T-Lt. Where T-Lt is the total number of T-L cells in whole blood and T-Lr is the number recovered in the final product. A representative blood smear from the treated individual is shown in FIG. 2.

The procedure yielding a T-L count of 22,400 cells/μL in whole blood and 27,060 cells/μL in the TLRP, corresponding to a 20.8% recovery of T-L cells and represented a 1.2-fold concentration (120.8%) relative the baseline. The prepared TLRP was stored frozen at −20 °C until use.

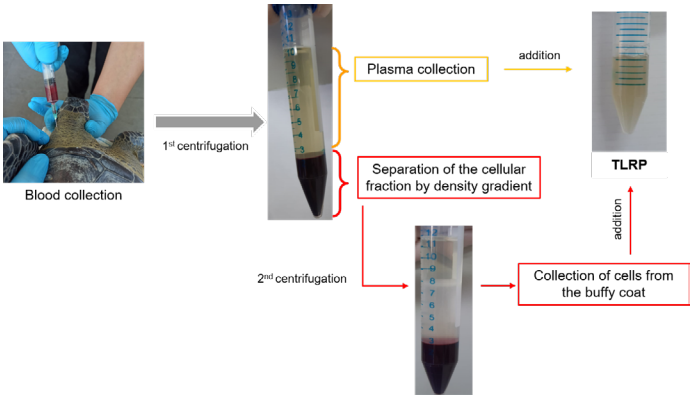

FIGURE 1. Schematic representation of the preparation of thrombocyte-leukocyte rich plasma (TLRP). The process includes blood collection, initial centrifugation, plasma separation, cellular fraction collection, and separation by density gradient to concentrate the inflammatory layer (buffy coat), ultimately resulting in TLRP formulation

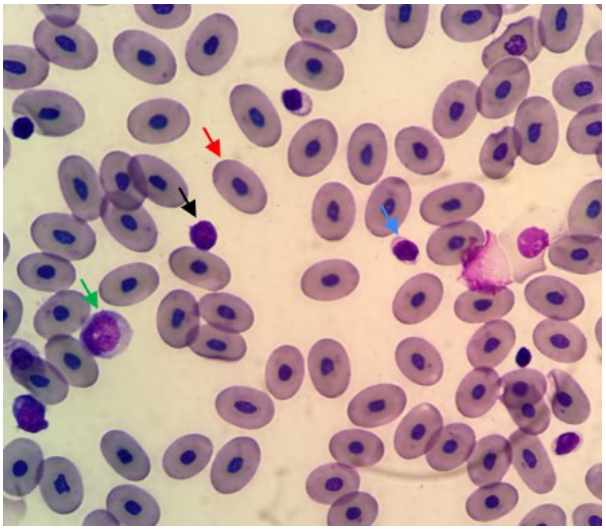

FIGURE 2. Microscopic image (1000×) of thrombocytes, leukocytes, and erythrocytes from the blood smear of the treated specimen used for the preparation of thrombocyte-leukocyte rich plasma (TLRP). Black arrow: lymphocyte; light blue arrow: thrombocyte; green arrow: monocyte; red arrow: erythrocyte

Therapeutic application of TLRP

A clinical case was selected involving cutaneous lesions and abscesses in the neck and forelimb regions of a green turtle undergoing rehabilitation at the Karumbé civil association (collection and capture permits: DINAMA-MVOTMA 4/2018; DINARA-MGAP 195/2018 and 50/2019). The most likely cause of the lesions was a skin wound that became secondarily infected. Such injuries are often the result of trauma caused by contact with rocks or abrasions sustained during stranding events. The turtle weighed 3.35 kg and measured 31 cm in standard curved length. Under general anesthesia, surgical debridement was performed to remove the abscesses, with each wound site cleaned to healthy margins. Anesthesia was induced with intravenous propofol (3–5 mg/kg) until the desired anesthetic depth was achieved. A 4.5 mm endotracheal tube was placed, and anesthesia was maintained with 3% isoflurane in a low-flow oxygen open-circuit system. Tramadol (3–5 mg/kg) was administered for analgesia.

To enable gelation and enhance retention at the application site, 10% (W/V) calcium chloride was added to the TLRP ten min prior to administration. The hemocomponent was injected perilesionally and intralesionally (FIG. 3).

In larger defects created by curettage, gelified TLRP was applied directly by allowing it to solidify within the syringe and then placing it into the wound bed, thus forming a three-dimensional matrix. Nylon film was used to wrap and retain the gelified TLRP at the application sites. Clinical follow-up included a one-month antibiotic therapy regimen with gentamicin at 6 mg/kg every 72 h, based on results from intralesional cultures. All procedures were conducted in accordance with the experimental protocol approved by the Ethics Committee (CEUA) of the Facultad de Veterinaria, Universidad de la República, Uruguay (Protocol No. 1421).

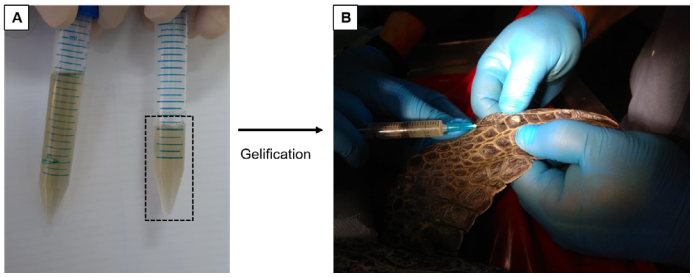

FIGURE 3. Therapeutic application of thrombocyte-leukocyte rich plasma (TLRP). A: TLRP prepared for application. B: Application of TLRP after activation with calcium chloride to achieve gelification, improving retention at the treatment site

[10 , 12 , 13], variations in T-L recovery are influenced by individual biological variability and methodological nuances, such as sample handling and centrifugation parameters.

However, the true therapeutic value of hemocomponents such as PRP and TLRP does not solely depend on cell concentration but also on the quality and viability of bioactive factors released during application. Although some studies in mammals suggest a possible dose-dependent effect of PRP, the optimal therapeutic concentration has not yet been clearly established in either mammals or reptiles [12 , 13 , 14]. Therefore, further studies are necessary to determine effective concentration thresholds and to correlate them with measurable regenerative outcomes in turtles and other reptile species.

Clinical case progression

Two days' post-treatment, improvement was observed in the ventral mandibular wounds. After two weeks, significant improvement was noted on the right side upon visual inspection, while the left side showed moderate improvement. Lesions on the forelimbs also improved, with the right-side lesion reappearing in smaller size compared to pre-treatment, and only minor abrasions remaining on the left side. A two-month follow-up confirmed continued healing progression with no signs of infection or adverse reactions (FIG. 4).

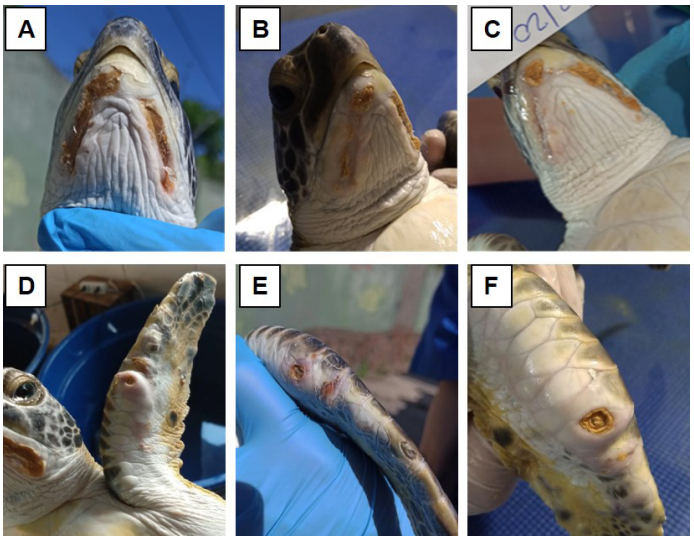

FIGURE 4. Clinical case: Presence and progression of cutaneous lesions. A–C: Mandibular lesions; D–F: Abscessed lesions in the left forelimb. A and D: Before treatment. B and E: 2 days post-treatment. C and F: 21 days post-treatment

RESULTS AND DISCUSSION

The recovery rates obtained in this study (20.8%) were lower than those reported by Di Ianni *et al.* [12], who documented a 48.9% recovery rate. This discrepancy could be attributed to differences in the resuspension volume used in the final formulation, which in their case achieved a 3–4× concentration of T-L cells relative to baseline. Despite this, using the same protocol, we previously obtained a 4× concentration in another clinical case involving *Chelonia mydas* (unpublished data), supporting the protocol's effectiveness in this species. As highlighted in both mammalian and herpetological literature

Traumatic injuries in turtles are among the most challenging and prolonged conditions to treat, often resulting in frustrating outcomes when healing fails. This reality has driven the search for alternative therapies, such as hemocomponents, which are emerging as safe and cost-effective treatment options. In this context, this study represents one of the first documented clinical applications of TLRP in a green turtle (*Chelonia mydas*). However, literature on TLRP and related hemocomponents in herpetological medicine remains scarce [10 , 12]. Nonetheless, the clinical case presented here showed a positive response to treatment, with enhanced wound healing. This aligns with

findings reported by other authors who have observed favorable outcomes following TLRP or similar hemocomponent therapy in turtles [7],[12] and in other reptile species [10 , 15 , 16]. However, despite these encouraging results, the study has some limitations, as it involved only a single clinical case and lacked precise quantitative assessment of wound healing during follow-up.

CONCLUSION

For the first time, the preparation of TLRP in *Chelonia mydas* was successfully reported. TLRP was applied effectively in a clinical case without adverse reactions and was associated with appropriate wound healing. To further elucidate its therapeutic effects, future studies should include a larger number of clinical cases and incorporate complete hematological and biochemical evaluations to better correlate physical recovery with the animal's physiological status

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this work.

Authors' contributions

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version

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