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SHORT REPORT

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A procedure using low-cost reagents to prepare allogeneic platelet gel from standard platelet units

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Abstract

Background and Objectives: Highly effective procedures for the preparation of allogeneic platelet gel (PG) use Ca-gluconate and batroxobin, an expensive commercial reagent. In this preliminary study, we explored the use of the plasmin-inhibitor, low-cost drug tranexamic acid (TXA) in place of batroxobin, based on the literature supporting TXA ability to prevent fibrinolysis and stabilize the gel formed by fibrin polymerization prompted by Ca-gluconate.

Materials and Methods: Eight blood centres determined PG weight and volume of non-gelled, liquid portion in 116 PG prepared in 20-mL commercial BioNest D bags. Ten-millilitre platelet aliquots from platelets in 100% plasma or in 35% plasma/65% platelet additive solution (PAS) were aseptically transferred into the D bag, followed by the injection of 3.3 mL of 10% Ca-gluconate and 0.4 mL of TXA. After 30-min incubation, PG weight and non-gelled liquid volume were determined.

Results: PG weight and liquid volume at 30 min were 6.5 ± 3.4 g and 7.4 ± 3.5 mL with platelets in 100% plasma, and 3.7 ± 3.0 g and 10.2 ± 3.3 mL with PAS platelets, respectively.

Conclusion: This study provides preliminary evidence supporting the use of TXA as a low-cost reagent for PG manufacturing from platelets in 100% plasma.

Keywords

batroxobin, platelet gel, tranexamic acid, wound healing

Highlights

- This study supports the use of Ca-gluconate and tranexamic acid for the preparation of allogenic platelet gel (PG).
- Platelets in 100% plasma were more efficient at producing PG than platelets in platelet additive solution.
- The weight of PGs decreases by 50% after 6 h, and therefore its use within a few hours is recommended.

INTRODUCTION

Platelet gel (PG) is a blood component used for non-transfusion applications, primarily in wound healing [1–3]. Although autologous blood platelets were initially favoured for PG preparation due to presumed higher microbiological safety, challenges such as repeated blood collection for paediatric and elderly patients requiring multiple applications, difficulties in autologous PG standardization, and the potential presence of pro-inflammatory factors in patients' blood have shifted focus to allogeneic sources. Advances in microbiological testing of allogeneic donor blood further supported this transition.

Allogeneic PG can be prepared by adding Ca-gluconate, with or without thrombin, to platelet aliquots obtained from standard platelet units collected from healthy blood donors [1, 2]. To improve gel formation, batroxobin—a 43-kDa protein derived from the venom of *Bothrops atrox*—can be used in place of thrombin [4]. Batroxobin significantly shortens gel formation time and enhances the gel's physical properties, but its high cost may limit its use in resource-constrained settings [5].

A potentially interesting alternative to batroxobin is the use of tranexamic acid (TXA), a synthetic lysine-analogue antifibrinolytic drug with multiple clinical applications [6]. This approach was inspired by preliminary literature evidence indicating that TXA's antifibrinolytic properties enhance the stability of gels formed through fibrin polymerization induced by Ca-gluconate [7, 8].

Platelets are essential for transfusion therapy in medical and surgical patients. However, they are perishable and can only be stored for 5–7 days at 20–24°C due to the risk of bacterial contamination. This limited shelf life can result in 10%–20% platelet wastage, contributing to the high costs of transfusion therapy [9]. Using standard platelet units originally prepared for transfusion but ultimately unused for this purpose offers a practical solution to increasing PG availability while reducing the wastage of this valuable resource. Due to the critical role of fibrinogen concentration in the formation of a firm and stable PG, we sought to investigate the physical characteristics of allogeneic PG prepared with Cagluconate and TXA from aliquots of standard platelets stored in 100% plasma ('high-fibrinogen' platelets) or in 35% plasma/65% platelet additive solution (PAS) ('low-fibrinogen' platelets).

This preliminary study belongs to a broader multi-centre project initiated by the Multicord12 network [10], with the aim of developing gelling procedures that rely on low-cost reagents.

MATERIALS AND METHODS

Platelet aliquots to manufacture PG were obtained from standard platelets prepared from buffy coat (BC) pools stored for 1–5 days in 100% plasma or in 35% plasma/65% PAS. Fibrinogen concentration in 'low-fibrinogen' platelets was estimated based on the percentage of plasma (35%) present in the original platelet unit.

Eight blood centres followed a common procedure to determine the PG net weight and the volume of non-gelled, liquid portion in 116 PG. Each centre, depending on the local availability of platelet units derived from BC pools, prepared a median of 15 PG (range 3–24). Platelet counts in the original platelet units were determined with a haematology automated cell counter (Sysmex XN, Norderstedt, Germany). The PG was prepared in BioNest D bags, which display a nominal volume of 20 mL and include a lateral 'easy opening' engraving facilitating PG removal and therapeutic application (BioNest 12D, White Nest Pharma, Milan, Italy).

Ten-millilitre platelet aliquots were aseptically transferred from a standard platelet unit to the D bag, followed by the injection of 3.3 mL of 10% Ca-gluconate and 0.4 mL of TXA (500 mg/5 mL, Bioindustria L.I.M., Novi Ligure, Italy; local price: 7.8 euro for five 5-mL vials) [8]. The BioNest D bag was incubated for 30 min at room temperature in horizontal position. Finally, the bag was opened using the easy-opening feature (Figure 1a) and the gel was removed with the aid of a Pasteur pipette (Figure 1b). Excess liquid was discarded to ensure accurate weighing of the PG. The gel was then left at room temperature for 6 h in a Petri dish (Figure 1c) and subsequently weighed again, with any excess liquid released from the PG being removed (Figure 1d).

The volume of non-gelled portion at 30 min (mL) was calculated as follows: Volume of aliquot + Ca-gluconate + TXA (mL) − (PG net weight at 30 min (g)/1.022).

Ethical committee approval was not required for this study, as the product was used exclusively for experimental purposes, with no tests involving human subjects. Moreover, no personal data related to blood donors were considered.

RESULTS

Platelet storage media, number of samples, platelet count in platelet aliquots, volume of aliquot + Ca-gluconate + TXA, volume of the non-gelled portion at 30 min, PG net weight at 30 min, and PG net weight at 6 h are reported in Table 1. PG prepared with 10-mL platelet aliquots from 'high-fibrinogen' platelet units stored in 100% plasma (representative example shown in Figure 1) showed a net weight of 6.5 ± 3.4 g and a volume of the liquid, non-gelled portion of 7.4 ± 3.5 mL after 30-min incubation. The PG weight decreased to approximately one half after 6 h. The use of platelet aliquots from 'low-fibrinogen' platelet units stored in PAS yielded smaller PG, weighing 3.7 ± 3.0 g and 1.9 ± 2.1 g at 30 min and 6 h, respectively.

DISCUSSION

This preliminary study aimed to develop a procedure using low-cost reagents to prepare allogeneic PG. The results, consistent with data from other systems [7, 8], support the use of TXA combined with Ca-gluconate to prepare allogeneic PG from standard, 'high-fibrinogen' platelet units stored in 100% plasma. In fact, the mean PG weight of 6.5 ± 3.4 g obtained from 10-mL platelet aliquots would be sufficient for topical applications in most diabetic foot ulcers, which are

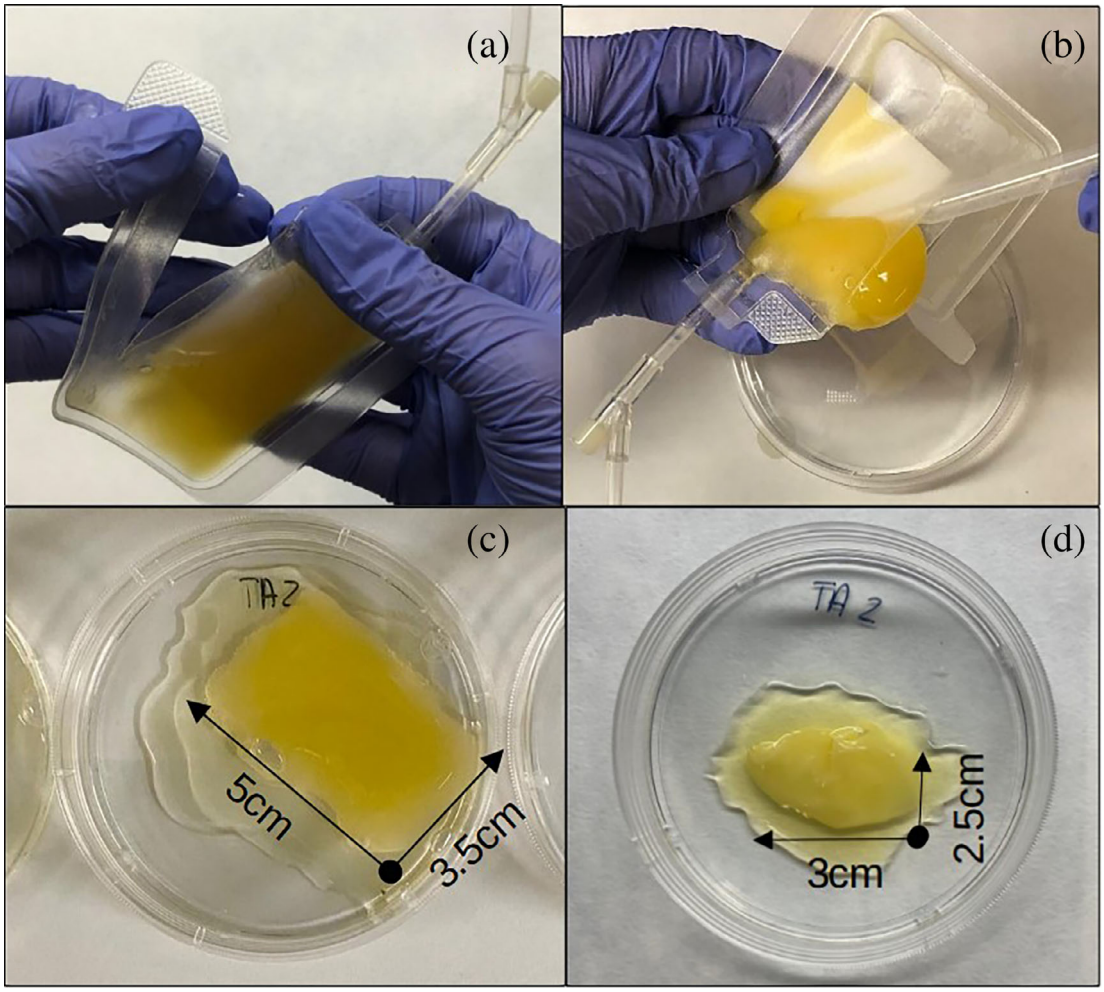

FIGURE 1 A representative example of a platelet gel (PG) prepared in a BioNest D bag, demonstrating its 'easy opening' feature, using a 10-mL platelet aliquot from a 'high-fibrinogen' platelet unit stored in 100% plasma (a). After a 30-min incubation, the PG was extracted from the BioNest D bag and transferred into a Petri dish (b), forming a gel with dimensions of 5 × 3 cm and a net weight of 6 g (c). Following 6 h of incubation at room temperature, the gel showed reduced dimensions of 3 × 2.5 cm and a net weight of 3 g (d).

TABLE 1 Results (mean ± SD) obtained with 10-mL platelet aliquots from standard platelets stored in 100% plasma or in 35% plasma/65% platelet additive solution.

Platelets storage media	No. of samples	Platelet count in platelet aliquots (10^9/L)	Volume of aliquot + Ca-gluconate + TXA (mL)	Volume of non-gelled portion at 30 min (mL)	PG net weight at 30 min (g)	PG net weight at 6 h (g)
100% plasma	57	1059 ± 271	14.1 ± 1.9	7.4 ± 3.5	6.5 ± 3.4	3.1 ± 2.0
35% plasma/65% PAS	59	886 ± 264	14.2 ± 3.2	10.2 ± 3.3	3.7 ± 3.0	1.9 ± 2.1

Abbreviations: PAS, platelet additive solution; PG, platelet gel; TXA, tranexamic acid.

smaller than 5 cm^2 in 75% of cases [11]. However, not surprisingly, lower PG weights were observed with 'low-fibrinogen' platelet units, which could be insufficient for the treatment of a proportion of ulcers. To resolve this limitation, PAS could be replaced with 100% plasma by centrifugation of platelet units prior to PG preparation in settings where PAS platelets, currently the standard platelet product prepared for transfusion in many jurisdictions, are the only available source. In addition, we observed that the gel weight decreased after 6 h. We analysed this time frame, where 6 h represents a worst-case scenario, because it provides more efficient logistics of PG therapeutic use in comparison with shorter intervals [5]. This finding highlights the importance of early PG application after activation.

Regarding the cost of the PG manufacturing reagents, the local market price of 7.8 euro for five 5-mL vials of the TXA used in this study translates into an expense of 1.56 euro for each vial used for the activation of one PG. This trivial cost, added to the average European Union (EU) market price of 1 euro or less per one 10-mL vial of 10% Ca-gluconate, makes the described procedure significantly less expensive in comparison with a literature report quoting a batroxobin cost of euro 84 per PG activation procedure [5].

Our study has limitations that will be addressed in future investigations. First, we did not determine the impact of the platelets' ABO blood group, storage duration before PG activation and procurement by apheresis. Second, we did not determine the optimal TXA volume for PG activation and detailed kinetics of PG weight reduction. Third, we did not evaluate the concentration of platelet-derived tissue regenerative factors entrapped into the PG and present in the non-gelled, liquid portion that would be mostly lost at the time of clinical application. Other future informative studies include evaluations of PG physical changes during storage [8].

Despite the growing interest in the clinical use of allogeneic PG, the lack of harmonized regulatory frameworks across international agencies limits high-quality trials to evaluate its efficacy in different conditions and the comparison of PG prepared with different methods. Encouragingly, the recent EU Regulation 2024/1938 on substances of human origin (SoHO) and initiatives like the GAPP-PRO Joint Action aim to standardize risk assessment and regulatory processes concerning these novel biological products within the EU [12].

In conclusion, this multi-centre study provides preliminary evidence supporting the use of Ca-gluconate and TXA as an economical approach to preparing allogeneic PG from 'high-fibrinogen' platelets stored in 100% plasma. Further research is needed to develop cost-effective procedures for PG production from 'low-fibrinogen' PAS-stored platelets and to fully evaluate their clinical efficacy in comparison with other procedures using more expensive reagents.

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L.M. and T.M. designed the study, performed the data analysis and wrote the manuscript, L.M., J.C., A.G., E.L., M.E.S., D.S. and G.S. performed the experiments and acquired the data, L.M., J.A.G.E. and T.M. prepared the draft version of the manuscript. All authors approved the manuscript. Open access funding provided by BIBLIOSAN.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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