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Use of clinical biological tests of haemostasis to evaluate topical haemostatics

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Abstract

Introduction: In addition to traditional means, topical haemostatics are currently used to avoid haemorrhage during surgery. Although they have been reported to be effective, there is a low level of proof of their clinical efficacy, which is at odds with their levels of use. This study used two methods to better understand their in vitro mechanism of action.

Methods: Two clinical biology assays were used to measure the action of topical haemostatics on primary and secondary haemostasis. Calibrated samples of collagen sponges and polypropylene non-woven gauze were tested. Platelet aggregation was assessed using a multichannel aggregometer. A thrombin generation assay (TGA) was used with a fluorogenic readout. Tissue factor solutions were used to activate coagulation.

Results: In terms of primary haemostasis, collagen sponges stimulated platelet aggregation, in particular between 2 and 5 min after incubation with platelet-rich plasma and with no dose effect. In regard to coagulation, the kinetics of thrombin generation was enhanced. Polypropylene non-woven gauze did not exhibit any effect on platelet aggregation, although it did have a weak effect on the kinetics of thrombin generation.

Conclusion: Collagen is well known to exert a haemostatic effect due to its action on platelet aggregation. By contrast, polypropylene non-woven gauze has not been shown to have any effect on platelet aggregation other than a minor impact on thrombin generation. The results obtained with the devices tested are in agreement with the literature. Platelet aggregation biological assays and TGA measurements appear to be suitable for evaluation of these medical products.

KEYWORDS

collagen sponge, haemostatics, platelet aggregation, thrombin generation, wound dressing

1 | INTRODUCTION

All abnormal bleeding occurring during surgery or postoperatively require haemostasis to be restored.¹ The risks for patients vary

Fabien Nativel and Sophie Tollec contributed equally to this work.

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depending on the patient, the anesthesia, the surgery,² and the timing of the onset of the bleeding. They can result in a longer operative duration, an increase in blood transfusion, and resumption of surgery, which generally lead to an increase in morbidity, mortality, and the costs for the facility.³

Over the past 60 years, numerous surgical haemostatic products have been marketed with the aim of becoming the ideal haemostatic agent. In addition to the qualities required for a haemostatic effect, haemostatic agents also need to (i) have structural components that allow for adequate platelet adhesion; (ii) activate coagulation; (iii) avoid fibrinolysis; (iv) be biodegradable when implanted; and (v) be made of biocompatible materials, as well as their degradation products.⁴ These range from conventional items such as alginate or cellulose pads to more recently developed items such as collagen and gelatin sponges. Synthetic glues were marketed in the 1990s, rapidly followed by fibrin glues, thereby resulting in an increase in reference products and overlapping indications. At present, there is still no surgical consensus as to the preferred haemostatic agent for different indications.

Surgical haemostatic agents comprise a large and heterogeneous class of products. They can be derived from a range of sources: vegetable (alginate, cellulose), animal (collagen, gelatin), synthetic (cyanoacrylate, aldehyde, polyethylene glycol), and human (fibrin glues, autologous fibrin). Their mechanisms of action in haemostasis vary and can be direct or indirect.^{5,6} These products can be divided into blood-derived drugs and medical devices (MD).⁷ Some are considered to be drugs based on their mode of action and composition (especially those incorporating components from human blood). In this case, authorization needs to be obtained to market them (AMM: Autorisation de Mise sur le Marché). Other products are considered to be MD, in which case they must have the CE marking. Claims by the manufacturers in terms of ownership and clinical use can lead to different classifications and, therefore, different assessments. Evaluation of the toxicity of haemostatic drugs is carried out by means of phase I and phase II clinical trials. MD are typically evaluated using *in vitro* and *in vivo* tests according to ISO EN 10993.^{8,9} Combined with other biocompatibility tests, the ISO 10993-4 guidelines provide methods to assess new biomaterials and MD. In addition, these tests provide information regarding the function and the mechanism of MD scanning with blood.^{10,11} Despite these differences, it is nonetheless clear that the immediate toxicity of surgical haemostatics is limited. Very little is known about chronic toxicity, which is mainly due to the limited number of studies and publications on this subject.

For several reasons, the efficacy of haemostatic agents is less obvious. This is due firstly to the lack of knowledge regarding their mechanism of action, with the exception of products that incorporate natural clotting factors. The second reason relates to the methodology of the clinical studies carried out to date. These have rarely been randomized comparative studies and they have mainly been limited to the bleeding duration. They have not measured the reduction of bleeding in terms of the volumes of blood transfusions or the reduction in the number of interventions or hospital stays.

However, in an environment that is increasingly subject to legal constraints, surgeons are not inclined to avoid using products that they consider to be ineffective but otherwise harmless.

This study aimed to determine the *in vitro* mechanism of action of topical haemostatics using two biological assays designed to study haemostasis.

2 | MATERIALS AND METHODS

2.1 | Blood collection

Blood was obtained from three healthy donors based on the following criteria: adult donor (age >18 years) with no history of hemorrhage or thrombosis and who had not taken any medication likely to modify haemostasis (e.g., antiplatelet agents, anticoagulants or hormones) in the 10 days prior to the blood collection.

Peripheral venous blood was collected into tubes (Vacuette[®], Greiner Bio-One, France) containing citrate (0.109 M, 9:1). The blood was carefully drawn to avoid unintentional platelet activation. The blood samples were centrifuged at 900 rpm for 10 min at room temperature to obtain platelet-rich plasma (PRP). To obtain platelet-poor plasma (PPP), PRP samples were centrifuged at 3500 rpm for 15 min. After counting the PRP using an automated cytology device, PRP were adjusted by dilution in PPP to $250 \times 10^9/L$ platelets for the aggregometry assays and to $150 \times 10^9/L$ platelets for the thrombin generation assays (TGA), as suggested by Yan et al.¹²

The PRP obtained were then pooled in order to obtain a sufficient volume of PRP for the two assays and to avoid any inter-individual variability.

2.2 | Optical aggregometry assays

Optical aggregometry assays were carried out on PRP in an eight-channel aggregometer (Soderel Medical[®], France) at 37°C. The potential for aggregation of the PRP was controlled by the addition of ristocetin solution (0.8, 1.2, and 1.5 mg/mL), adenosine diphosphate-ADP (2.5, 5.0 μ M), epinephrine (2.0 μ M), arachidonic acid (1.6 mM), and collagen (5.0 μ g/mL).

Samples with surface areas of 28 mm² were obtained using a hole punch on collagen dressings composed of lyophilized non-denatured native bovine collagen (Pangen 2[®], Urgomedical, France) or non-woven gauze (Tetra Medical, France) and then incubated in 1 mL of PRP for 30 s, 1 min, 2 min, 3 min, 4 min, 5 min, or 10 min. The number of samples added to the PRP was increased from 1 to 3 to evaluate the platelet aggregation effect. PRP alone, incubated in the same conditions, was used as a control for the absence of spontaneous aggregation.

After incubation, a 300 μ L aliquot of PRP was withdrawn and placed in a disposable plastic vessel containing a stirring bar.

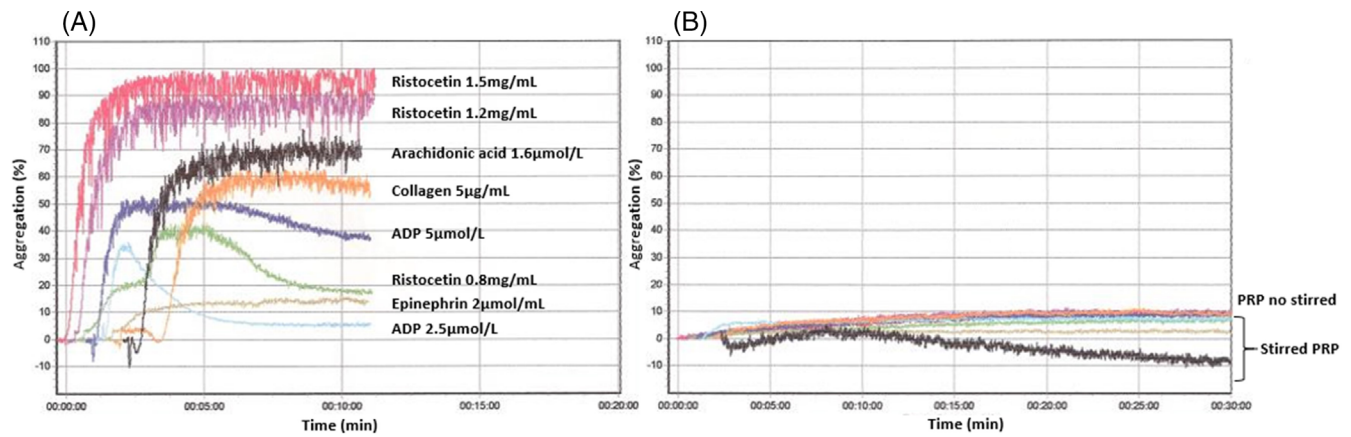


FIGURE 1 Platelets aggregometry of PRP measured by optical test in healthy donors. PRP show aggregation in the presence or absence of platelets inducers. (A) Aggregation is over 80% with ristocetin and over 50% with collagen, ADP, and arachidonic acid. (B) No platelets aggregation was observed during 30 min without inductor. PRP stirring time decreased % aggregation (t: 30 s–10 min). ADP, adenosine diphosphate; PRP, platelet-rich plasma.

2.3 | Thrombin generation assays

Thrombin generation was measured in PRP using a fluorogenic method as previously described.¹³ Samples of collagen dressing (Pangen 2[®]) and non-woven gauze (Tetra Medical, France) were placed in 96-well plates. The size of the samples (surface area = 32 mm²) was such that they covered the bottom of the wells. An 80 µL aliquot of PRP was added to each well. PRP without material was used as a negative control. A 20 µL aliquot of tissue factor (TF, 0.5 pM) was added to the PRP. Thrombin generation was measured at 37°C using a CAT System[®] (Stago, Asnières sur Seine, France) after adding 20 µL of a solution containing CaCl₂ and the fluorogenic substrate. HEPES buffered saline (HBS, Sigma-Aldrich, France) was used as a control for the absence of spontaneous coagulation of the PRP.

The lag time (LT), the endogenous thrombin potential (ETP), the thrombin peak (TP), and the time to peak (TTP) were determined for each incubation time. The measurements usually lasted 60 min. All of the experiments were carried out in triplicate.

3 | RESULTS

3.1 | Optical aggregometry assays

Under these experimental conditions, platelet aggregation was obtained by using agonists in the same manner as in clinical use (Figure 1A). As expected, there was no aggregation over the course of a 30 min period when the PRP was tested without any agonist (Figure 1B). PRP incubated with the non-woven gauze ($n = 3$ samples) did not exhibit platelet aggregation over a 30 min period with any of the agonists (Figure 2A). No aggregation was observed after samples of collagen dressing were incubated in PRP, irrespective of the number of samples, when the pre-analysis incubation was <2 min

(Figure 2B–D). Surprisingly, no dose-dependent effect was observed when the number of samples of collagen dressing incubated in PRP was increased. When platelet aggregation occurred, it began immediately after introduction of the agonist and it was complete after 5 min.

3.2 | Thrombin generation assays

The results of the TGA are shown in Figure 3. The use of a TF solution at 0.5 pM induced thrombin generation with a LT of 5 min, an ETP of 2454 nM min, a TP of 123 nM, and a TTP of 18 min. With polypropylene non-woven gauze and without TF addition, the results were a LT of 7 min, an ETP of 2149 nM min, a TP of 140 nM, and a TTP of 10 min. The use of collagen resulted in a clear increase in thrombin generation (LT = 7 min, ETP = 2690 nM.min, TP = 421 nM, TTP = 10 min) compared to polypropylene non-woven gauze. Using only the HBS buffer, spontaneous thrombin generation was delayed (LT = 17 min, ETP = 1776 nM min, TP = 89 nM, and TTP = 24 min).

4 | DISCUSSION

Interest in haemostatic agents has increased over the past years for several reasons. The first is linked to the resurgence of armed conflict and the need to provide effective haemostasis in a timely manner in order that survival following injury is not dependent on the evacuation time. The nature of combat injuries differs from those seen in civilian medical facilities due to the complex nature of the battlefield environment, the severity of the injuries, and the concomitant manifestation of multiple pathophysiological disorders that delay the healing process.^{14,15} The second reason is related to the quest for a reduction of operative morbidity with reduced length of surgery, transfusions, and iatrogenic surgery complications.¹⁶

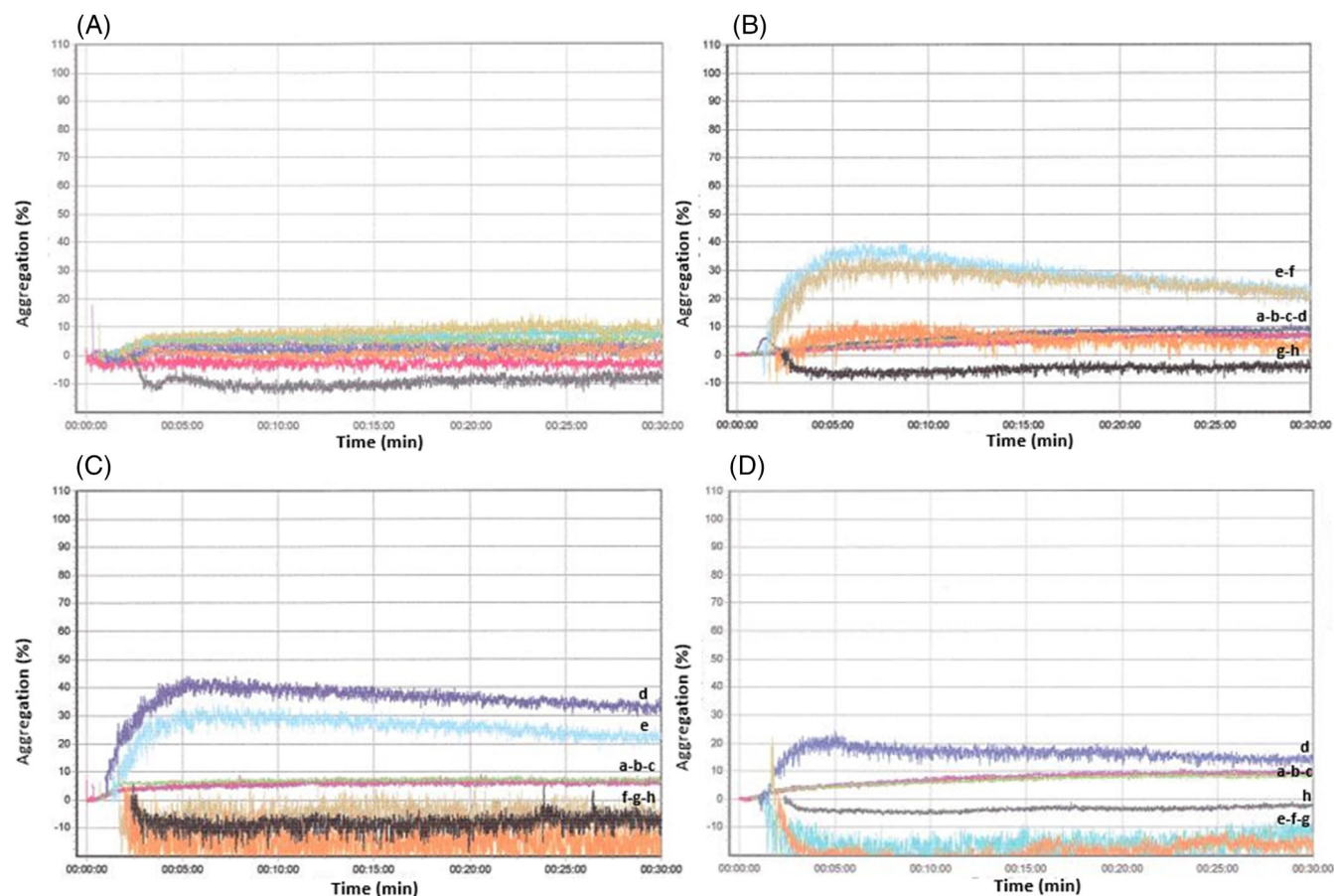


FIGURE 2 Platelets aggregometry of platelet-rich plasma (PRP) incubated with non-woven gauze or collagen dressing measured by optical test in healthy donors. (A) No platelet aggregation was observed during 30 min with PRP incubated first with three samples of non-woven gauze. (B) Aggregation (>30%) is obtained after incubation of one sample of collagen dressing. (C) Aggregation (>30%) is obtained after incubation of two sample of collagen dressing. (D) Aggregation (>20%) is obtained after incubation of three sample of collagen dressing. Time of incubation: (a) $t = 0$ s; (b) $t = 30$ s; (c) $t = 1$ min; (d) $t = 2$ min; (e) $t = 3$ min; (f) $t = 4$ min; (g) $t = 5$ min; (h) $t = 10$ min.

The availability of new commercial products (drugs or DM) has increased in recent years, with three main criteria: (i) usability, (ii) stability, and (iii) biocompatibility. The last aspect determines their mechanism of action, kinetics, and intensity. Hemostatic assessments are mainly carried out *in vivo* in animal or clinical studies, focusing on biological parameters such as hematocrit, the amount of blood lost or transfused, and the number of re-interventions.

Using platelet aggregation assays and TGA, this study aimed to provide a better understanding of the *in vitro* mechanism of action of haemostatic agents.

This approach meets the requirements of NF EN ISO 10993. Although this standard is not applied to drugs, *in vitro* evaluation can serve as a worthwhile preclinical tool for haemostatic MD and drugs.

Platelets are the smallest blood cells (1–3 μm) and they are present in large quantities in blood (1.5–3.5 $\times 10^5$ cells/ μL). Platelets have a critical role in haemostasis after vessel injury. The process of platelet plug formation consists of a successive process of platelet adhesion, activation, and aggregation.^{17,18} For haemostatic agents to be effective, they need to act on these events. A number of *in vitro* studies have focused on the interactions between platelets and

haemostatic materials, by studying platelet adhesion by an indirect method based on measurement of the lactate dehydrogenase enzyme released by adhered platelets after lysis,¹⁹ by using flow cytometry with PAC-1-FITC reagent and by using fluorescence microscopy with static microarrays,²⁰ or by studying platelet activation such as platelet factor 4 (PF4) using ELISA²¹ and by serotonin analysis using chromatography.²² Assessment of just the adhesion phase is of limited relevance because numerous biomaterials enhance platelet and cell adhesion without exerting haemostatic properties. By contrast, an effect on aggregation is enough to warrant assessment of an entity's haemostatic potential. Aggregation assays therefore appear to be more suitable for haemostatic evaluation as they reflect the entire process of primary haemostasis.^{23,24} The most commonly used method for studying platelet aggregation of blood involves photometric measurement of PRP. This test is based on measurement of the change in optical transmission due to sedimentation of PRP exposed to inducers of aggregation. An aggregation of 100% equates to the optical transmission of a plasma sample for which the platelets have been removed by centrifugation (PPP). This biological test was altered for the objectives of this study, as it was not possible to introduce

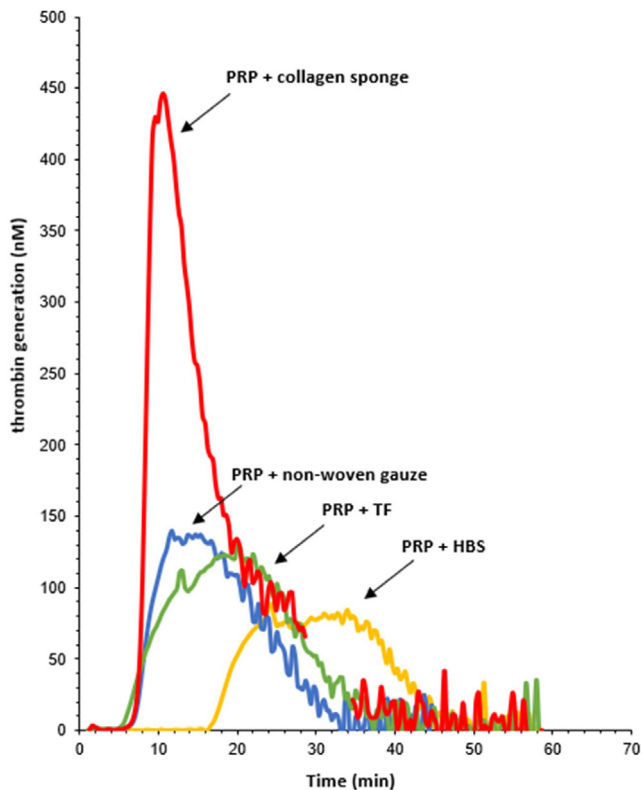


FIGURE 3 Thrombin generation measured by CAT System[®]. PRP + HBS (yellow); PRP + TF (green); PRP + non-woven gauze (blue); PRP + collagen sponge (red). Peak of thrombin is higher in PRP incubated with non-woven gauze samples than PRP with HBS. Peak of thrombin is faster and higher in PRP incubated with sample of collagen sponge than PRP with thrombin inductor. HBS, HEPES buffered saline, used to assess spontaneous coagulation; PRP, platelet-rich plasma; TF, tissue factor.

haemostatic agents directly into the measuring vessel of the spectrophotometer as this would have caused optical interference. Indeed, in whole blood, red blood cells prevent platelets from being deposited on the recording electrode. The evaluation of effective platelet participation is hence underestimated. In 2010, Segal et al noted an *in vitro* latency time of more than 5 min before detecting a variation of impedance indicative of a platelet response in citrated and heparinized whole blood.²⁵ The PRP was hence first incubated with samples of hemostatic agents before being transferred to the measuring vessel. Two materials were tested to validate the method: collagen dressing, which is a natural inducer of primary haemostasis, and non-woven gauze. The latter device is composed of polypropylene material that should theoretically have no effect on haemostasis and for which no clinical effect has been described. Both materials were added to the PRP for periods ranging from 1 to 10 min to record the kinetics of their effect. To ensure reproducibility of the measurements, the samples of the devices were calibrated. The surface area of the materials appears to be a better criterion than their weight because *in vivo* effects depend more on the contact area with the tissue than on the amount of material.

In keeping with its mechanism of action, the collagen dressing resulted in aggregation. Clearly, our methodological conditions do not allow for complete platelet aggregation (>70%) as is routinely observed with the use of potent platelet aggregating inducers (as illustrated in Figure 2). Thus, we arbitrarily chose the threshold of 30% platelet aggregation, which, in light of our results, seems relevant to differentiate topical haemostatic agents that had no effect on platelet aggregation from those that had a discernible effect. In this study, the effect of collagen was maximal when a calibrated sample was used (28 mm²/1 mL PRP), and it did not increase by doubling/tripling the amount of material. This could be explained by pre-activation of platelets causing the release of platelet granule contents and a reduced optical response. The collagen platelet aggregation curves appear to be comparable to those obtained with inducers such as ristocetin and ADP. This assay allows the action of haemostatic agents on platelet aggregation to be detected. These results are consistent with the purported action of collagen, which is thought to interact with platelet receptors (GPIa/IIa) and to thereby cause adhesion, activation, and aggregation with the fibrinogen present in the PRP.^{26,27} This aggregation assay can be applied directly to all haemostatic agents.

The TGA examines the overall haemostatic process leading to the formation of thrombin. The generation of thrombin *in vivo* is highly dependent on platelet procoagulant and consequently on their degree of function. Using this *in vitro* method with PRP and haemostatic agents allows the ability of these agents to act as TF to be evaluated. Dargaud et al showed the relevance of measuring thrombin generation on PRP and they demonstrated a dose-dependent increase of both the peak thrombin and the amount of thrombin produced in PRP upon the addition of increasing amounts of TF, with a high variability up to 25% at 1 pM of TF.²⁸ Use of a Thermo Electron fluorometer allows continuous measurement of thrombin formation.²⁹ Using PRP without addition of an inducer (TF) or without haemostatic agents allowed measurement of the time required to obtain spontaneous thrombin formation. Any agent that does not result in the formation of thrombin during this period can be considered to be non-inducer. Conversely, the addition of TF in PRP is a way to assess the ability of plasma to clot under the operating conditions and the minimum time to obtain this state. Haemostatic agents can act on thrombin generation and can be compared to the effect of TF. The thrombin generation measurements showed that polypropylene non-woven gauze had activity. Although the ETP was slightly lower (−10%) and the TP slightly higher (+10%) than that of TF, the overall extent of thrombin generation appeared to be similar in both conditions, as previously reported by Stief et al.³⁰ The collagen dressing acted faster than the polypropylene non-woven gauze and the TF. These results can be explained by the fact that the TF was not used at its maximum concentration and because PRP retains a capacity for thrombin formation.²⁸ However, this TF concentration was preferable because, as demonstrated by Dargaud et al low concentrations (<1 pM) allow for better assessment of haemostatic effects, especially for haemorrhagic disorders. The TGA is thought to be the most powerful method to assess *in vivo* haemostasis. Our results with collagen dressing

demonstrated better in vitro thrombin generation and could suggest a greater in vivo haemostatic effect.

The limitations of this test to study haemostatic agents are as follows: it lacks reproducibility for absolute quantification due to the variations in thrombin generation that can be generated between the blood samples from different donors. It, therefore, quantifies a relative procoagulant activity of haemostatic agents. Both of the materials that were tested were shown to have the capacity to generate thrombin. This thrombin generation, which occurred after a short LT, is unlikely to be an artefact. Although spontaneous thrombin generation, probably resulting from contact phase activation, is known to occur, this takes place significantly later (LT = 17 min) than what was observed with both dressings or by the use of TF. "Spontaneous" thrombin generation starts at the end of the "dressing- or TG-induced" generation phase. We are of the opinion that it does not affect our results. The use of corn trypsin inhibitor should have inhibited this contact-phase thrombin generation but, unfortunately, such sampling conditions were not available in our study. Applying this method to other agents may be of relevance for the identification of substances without activity and to compare them using the kinetics of thrombin generation and the areas under the curve. This assay suffers from another limitation that has not been observed in these experiments, which was that these methods do not work for agents that contain or that are composed of biological glues. Thrombin formation is obtained immediately during the incubation phase and then consumed before the beginning of the test, and the plasma can then no longer coagulate. The application of these biological assays under these conditions can demonstrate that collagen and non-woven gauze dressings have different actions on haemostasis. In vivo, collagen acts initially on primary haemostasis and can thus promote secondary coagulation. It can rapidly trigger the formation of a platelet clot and this ability to generate thrombin enhances clot formation and, therefore, the maintenance of haemostasis. Conversely, the lack of haemostatic action in vivo of non-woven gauze is explained by an inability to act on platelet aggregation even though our results suggest that the generation of thrombin can be stimulated in vitro. How this non-woven gauze could initiate thrombin generation without a procoagulant effect on platelets cannot be explained from our results.

These methods can be readily implemented as the required equipment is widely available and they are inexpensive compared to animal testing or clinical studies. These methods are reproducible in the sense that the results were comparable irrespective of the donor. However, they are sensitive to variation in the degree of coagulation between individual donors, and because the plasma requirements become excessive. The results of Dargaud et al could lead to better options, to the extent that they show the potential of using the thrombin generation test with blood obtained from mice.³¹

5 | CONCLUSION

This work highlights the multitude of families and galenic forms of surgical haemostatic agents available to users. Validated indications by

the AMM for blood-derived drugs and the CE mark for MD have undergone scant confirmation by clinical evaluations with a high level of scientific evidence. That is why, in this study, the use of in vitro models showed that it was possible to use two haemostatic criteria: the degree of platelet aggregation and the degree of thrombin generation, to rapidly determine the mechanism of action of haemostatic agents. Good surgical haemostasis practice is a local as well as a national issue. Indeed, the French National Authority for Health (HAS) ought to make national guidelines available in this regard. The addition of further haemostatic entities to the already existing therapeutic arsenal would be of great value if they could be shown to meet a particular need and at a reasonable cost, as confirmed by medico-economic studies (reduction of the incidence of blood transfusion and the duration of hospitalization).

AUTHOR CONTRIBUTIONS

All authors provided separate parts of the data. All of them read, corrected, and approved the submitted manuscript. **ST**: Performed all of the experiments. **ST, FN, MT**: Analysed the data. **FN**: Wrote the manuscript. **GG**: Supervised the work and conducted a critical read of the manuscript. **MT, OS**: Reviewed the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict 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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REFERENCES

- Zimmerman LH. Causes and consequences of critical bleeding and mechanisms of blood coagulation. *Pharmacotherapy*. 2007;27(9 Pt 2): 455-565. doi:10.1592/phco.27.9part2.455
- Hammond KL, Margolin DA. Surgical hemorrhage, damage control, and the abdominal compartment syndrome. *Clin Colon Rectal Surg*. 2006;19(4):188-194. doi:10.1055/s-2006-956439
- Stokes ME, Ye X, Shah M, et al. Impact of bleeding-related complications and/or blood product transfusions on hospital costs in inpatient surgical patients. *BMC Health Serv Res*. 2011;11(1):135. doi:10.1186/1472-6963-11-135
- Khoshmohabat H, Paydar S, Kazemi HM, Dalfardi B. Overview of agents used for emergency hemostasis. *Trauma Mon*. 2016;21(1): e26023. doi:10.5812/traumamon.26023
- Pereira BM, Bortoto JB, Fraga GP. Topical hemostatic agents in surgery: review and prospects. *Rev Col Bras Cir*. 2018;45(5):e1900. doi:10.1590/0100-6991e-20181900
- Hickman DA, Pawlowski CL, Sekhon UDS, Marks J, Gupta AS. Biomaterials and advanced technologies for hemostatic management of bleeding. *Adv Mater*. 2018;30(4):1700859. doi:10.1002/adma.201700859
- Aubourg R, Putzolu J, Bouche S, et al. Surgical hemostatic agents: assessment of drugs and medical devices. *J Visc Surg*. 2011;148(6): e405-e408. doi:10.1016/j.jviscsurg.2011.10.001
- Aydemir Sezer U, Kocer Z, Sahin İ, Aru B, Yanıkkaya Demirel G, Sezer S. Oxidized regenerated cellulose cross-linked gelatin

- microparticles for rapid and biocompatible hemostasis: a versatile cross-linking agent. *Carbohydr Polym*. 2018;200:624-632. doi:10.1016/j.carbpol.2018.07.074
9. Bernard M, Jubeli E, Pungente MD, Yagoubi N. Biocompatibility of polymer-based biomaterials and medical devices - regulations, in vitro screening and risk-management. *Biomater Sci*. 2018;6(8):2025-2053. doi:10.1039/c8bm00518d
 10. Stang K, Krajewski S, Neumann B, et al. Hemocompatibility testing according to ISO 10993-4: discrimination between pyrogen- and device-induced hemostatic activation. *Mater Sci Eng C Mater Biol Appl*. 2014;42:422-428. doi:10.1016/j.msec.2014.05.070
 11. Weber M, Steinle H, Golombek S, et al. Blood-contacting biomaterials: in vitro evaluation of the hemocompatibility. *Front Bioeng Biotechnol*. 2018;6:99. doi:10.3389/fbioe.2018.00099
 12. Yan Q, Ninivaggi M, de Laat B, de Laat-Kremers RMW. Reference values for thrombin dynamics in platelet rich plasma. *Platelets*. 2021;32(2):251-258. doi:10.1080/09537104.2020.1742310
 13. Hemker HC, Béguin S. Thrombin generation in plasma: its assessment via the endogenous thrombin potential. *Thromb Haemost*. 1995;74(1):134-138.
 14. Gruen RL, Brohi K, Schreiber M, et al. Haemorrhage control in severely injured patients. *Lancet Lond Engl*. 2012;380(9847):1099-1108. doi:10.1016/S0140-6736(12)61224-0
 15. Champion HR, Bellamy RF, Roberts CP, Leppaniemi A. A profile of combat injury. *J Trauma Acute Care Surg*. 2003;54(5):S13-S19. doi:10.1097/01.TA.0000057151.02906.27
 16. Choron RL, Hazelton JP, Hunter K, et al. Intra-abdominal packing with laparotomy pads and QuikClot™ during damage control laparotomy: a safety analysis. *Injury*. 2017;48(1):158-164. doi:10.1016/j.injury.2016.07.033
 17. Steinhubl SR, Moliterno DJ. The role of the platelet in the pathogenesis of atherothrombosis. *Am J Cardiovasc Drugs Drugs Devices Interv*. 2005;5(6):399-408. doi:10.2165/00129784-200505060-00007
 18. Clemetson KJ. Platelets and primary haemostasis. *Thromb Res*. 2012;129(3):220-224. doi:10.1016/j.thromres.2011.11.036
 19. Ong SY, Wu J, Moochhala SM, Tan MH, Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. *Biomaterials*. 2008;29(32):4323-4332. doi:10.1016/j.biomaterials.2008.07.034
 20. Gu R, Sun W, Zhou H, et al. The performance of a fly-larva shell-derived chitosan sponge as an absorbable surgical hemostatic agent. *Biomaterials*. 2010;31(6):1270-1277. doi:10.1016/j.biomaterials.2009.10.023
 21. Mayer A, Vadon M, Rinner B, Novak A, Wintersteiger R, Fröhlich E. The role of nanoparticle size in hemocompatibility. *Toxicology*. 2009;258(2-3):139-147. doi:10.1016/j.tox.2009.01.015
 22. Krízová P, Másová L, Suttnar J, et al. The influence of intrinsic coagulation pathway on blood platelets activation by oxidized cellulose. *J Biomed Mater Res A*. 2007;82(2):274-280. doi:10.1002/jbm.a.31060
 23. Elalamy I, Gkalea V, Gerotziapas G, Ketatni H, Hatmi M. The usefulness of platelet function evaluation in clinical practice. *Ann Biol Clin (Paris)*. 2013;71:47-57. doi:10.1684/abc.2013.0905
 24. Hvas AM, Favaloro EJ. Platelet function analyzed by light transmission aggregometry. *Methods Mol Biol*. 2017;1646:321-331. doi:10.1007/978-1-4939-7196-1_25
 25. Segal HC, Hunt BJ, Gilding K. The effects of alginate and non-alginate wound dressings on blood coagulation and platelet activation. *J Biomater Appl*. 1998;12(3):249-257. doi:10.1177/088532829801200305
 26. Coller BS, Beer JH, Scudder LE, Steinberg MH. Collagen-platelet interactions: evidence for a direct interaction of collagen with platelet GPIIb/IIIa and an indirect interaction with platelet GPIIb/IIIa mediated by adhesive proteins. *Blood*. 1989;74(1):182-192.
 27. Surin WR, Barthwal MK, Dikshit M. Platelet collagen receptors, signaling and antagonism: emerging approaches for the prevention of intravascular thrombosis. *Thromb Res*. 2008;122(6):786-803. doi:10.1016/j.thromres.2007.10.005
 28. Dargaud Y. Evaluation of a standardized protocol for thrombin generation measurement using the calibrated automated thrombogram: an international multicentre study. *Thromb Res*. 2012;130(6):929-934. doi:10.1016/j.thromres.2012.07.017
 29. Dargaud Y, Luddington R, Baglin T. Platelet-dependent thrombography: a method for diagnostic laboratories. *Br J Haematol*. 2006;134(3):323-325. doi:10.1111/j.1365-2141.2006.06188.x
 30. Stief TW. Influence of the surface on thrombin generation. *Int J Lab Hematol*. 2008;30(4):269-277. doi:10.1111/j.1751-553X.2007.00959.x
 31. Dargaud Y, Spronk HMH, Leenders P, Hemker HC, Ten Cate H. Monitoring platelet dependent thrombin generation in mice. *Thromb Res*. 2010;126(5):436-441. doi:10.1016/j.thromres.2010.08.007

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