Plasmatic and cell-based enhancement by microparticles originated from platelets and endothelial cells under simulated in vitro conditions of a dilutional coagulopathy

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Abstract

**Background:** Aggressive fluid management and other external factors may lead to hypothermia, acidosis and hemodilution (defined as Lethal Triad, LT) contributing to a trauma-induced coagulopathy (TIC) that worsens patients’ outcomes. Procoagulant microparticles (MP) are crucial players at the interface of cellular and plasmatic coagulation. However, their functions remain largely unexplored. This study aimed to characterize effects of MP subtypes and concentrations on functional coagulation under *in vitro* simulated conditions.

**Methods:** Blood from eleven volunteers were collected to simulate *in vitro* conditions of hemodilution (HD) and LT, respectively. HD was induced by replacing a blood volume of 33% by crystalloids and for LT, samples were further processed by reducing the temperature to 32 °C and lowering the pH to 6.8. MP were obtained either from platelet concentrates (platelet-derived MP, PDMP) or from cell culture (ECV304 cells for endothelial-derived MP, EDMP) by targeted stimulation. After introducing MP to *in vitro* conditions, we measured their concentration-dependent effects (1.000, 10.000 and 15.000 MP/µl blood) on coagulation compared to whole blood (WB). For each condition, coagulation was characterized by flow cytometric platelet activation and by quantification of fibrin clot propagation using Thrombodynamics® technology.

**Results:** MP originated from platelets and endothelial cells affected blood coagulation in a concentration-dependent manner. Particularly, high PDMP quantities (10.000 and 15.000 PDMP/µl blood) significantly induced platelet activation and fibrin clot growth and size in HD conditions. In LT conditions as well, only high PDMP concentration induced platelet activation, clot growth and size. In contrast, EDMP did not induce platelet activation, but resulted in enhanced formation of spontaneous clots, irrespective of simulated condition. With increasing EDMP concentration, the time until the onset of spontaneous clotting decreased in both HD and LT conditions.

**Discussion:** The study demonstrates an essential role of MP within the coagulation process under simulated coagulopathic conditions. PDMP affected platelets promoting clot formation likely by providing a surface enlargement. EDMP presumably affected clotting factors of the plasmatic coagulation resulting in an increased formation of spontaneous clots.

**Conclusion:** Under simulated conditions of a dilutional coagulopathy, MP from different cellular origin indicate a divergent but both procoagulant mechanism within the coagulation process.

Introduction

Despite continuous improvements in trauma management, traumatic injuries are still the leading cause of death and disability in adults under 40 years [1–3]. Particularly, uncontrolled bleeding contributes to more than 50% of all trauma-related deaths. The bleeding phenotype is significantly aggravated by a trauma-induced coagulopathy (TIC) occurring within hours after injury [4,5]. Approximately, one of four trauma patients arrive at the emergency department with laboratory signs of a compromised coagulation resulting in a four-fold higher mortality [6–8]. Efforts to elucidate the underlying pathomechanism led to improved resuscitation strategies in trauma management over the last decade, but still remain unknown in decisive parts [9,10]. Looking at etiology, the described mechanisms are currently divided into either a trauma and/or traumatic shock-induced endogenous coagulopathy (including endotheliopathy) also described as acute traumatic coagulopathy (ATC) or iatrogenic coagulopathy (IC) [3,11,12]. IC is triggered by an aggressive trauma and in particular fluid management that may lead to hypothermia, acidosis and hemodilution, which worsens the outcomes of severely injured patients significantly [12]. Due to the high impact on coagulation of these three external conditions, they are most recently referred as “lethal triad” (LT) although hemodilution was not part of the historical definition. A progressive course of LT is associated with a deterioration of coagulation accompanied by disturbed clot formation and strength [13–15]. For primary hemostasis and particularly platelet function under clinical conditions of ATC, a significant decrease in platelet activation and aggregation leading to deterioration of clot formation was described [16,17]. But in contrast to this mechanism, functioning at this boundary of cellular- and plasmatic-driven coagulation, small cell-derived subcellular
vesicles defined by size of 0.1 - 0.9 µm (known as microparticles, MP) are produced and released in large quantities from
different cell types [18–21]. MP therefore are mediators of the cellular and plasmatic coagulation and interact with various
systemic inflammatory pathways initiated after trauma and other acute-phase conditions (e.g. ARDS, [22] and multiple
organ failure [21,23]). There is evidence that different MP phenotypes trace back their cellular origin depending on their
membrane antigen composition. In addition, after major trauma, a characteristic distribution pattern correlating with injury
severity could be shown [24,25]. This study aimed to characterize effects of MP subtypes and concentrations on functional
coaulation under \textit{in vitro} simulated coagulopathic conditions. In relation to their potential to facilitate coagulation
complexes and initiate the coagulation process via TF-/FVII-dependent and independent pathways, our goal was to
understand and to differentiate the role of platelet- and endothelial-derived MP (PDMP, EDMP) on functional coagulation
[26,27]. Increasing MP concentrations were used to investigate their impact on primary and plasmatic hemostasis under
simulated standardized \textit{in vitro} IC-conditions.

\section*{Methods}

\subsection*{Targeted \textit{in vitro} synthesis of microparticles}

\subsubsection*{Cell line and cultivation for EDMP production}

Human ECV304 cells (Sigma-Aldrich, Steinheim, Germany) were used to investigate endothelial MP generation properties.
Cultivated in Dulbecco's Modified Eagle Medium (DMEM) and incubated under constant conditions at 37 °C, 5 % CO$_2$
and 90 % humidity, cells were subcultured by trypsinization (0.25 % trypsin, Sigma-Aldrich, Steinheim, Germany).

For generating particles, ECV304 cells were incubated with 1 mM hydrogen peroxide (Sigma-Aldrich, Steinheim, Germany);
platelets with 1.5 µg/ml bacterial lipopolysaccharide (LPS) (Sigma-Aldrich, Steinheim, Germany) – both for 22 hours at
37 °C maintaining established cultivation conditions. Subsequently, a first centrifugation step was performed to remove
cells (10 min at 1.000 x g, 4 °C) followed by a second step to sediment particles from transferred supernatant (45 min at
10.000 x g, 4 °C). After removing residual supernatant, pellets were resuspended in 1x phosphate buffered saline (PBS),
pooled and stored at -80 °C until analysis.

\subsubsection*{Platelet concentrates and PDMP production}

Human platelets were obtained from platelet apheresis concentrates of the Institute of Transfusion Medicine (ITM) of the
Cologne-Merheim Medical Centre that were not being authorised for transfusion.

For \textit{in vitro} synthesis of PDMP, 40 mls of platelet concentrates were stimulated with 1.5 µg/ml LPS for 22 h at 37 °C in a
humidified incubator with 5 % CO$_2$ (ThermoFisher, Marietta, USA). Subsequently, the platelet suspension was centrifuged at
1.000xg for 10 min at 4 °C (Heraeus, Hanau, Germany). Cell-free supernatant was centrifuged twice at 10.000xg for 45 min
at 4 °C (Heraeus, Hanau, Germany) for PDMP pellet sedimentation, which was resuspended in the following in PBS
obtaining pure PDMP. PDMP pellets were pooled, aliquoted and stored at -80 °C until analysis.

\subsection*{Blood donation and processing for inducing coagulopathic conditions}

Study approval was given by the ethical committee of Witten/Herdecke University (#182/2016). Eleven healthy volunteers,
fulfilling the ITM criteria for blood donation (age \textgreater= 18 years; no preexisting history of coagulation disorders, anticoagulant
and/or platelet-inhibiting medication or viral infection), consented to participate and donated 60 mls blood, which was
collected in citrated monovettes (Sarstedt, Nümbrecht, Germany).

Coagulopathic conditions such as haemodilution (HD) and lethal triad (LT) were simulated \textit{in vitro} as previously described
[28,29]. In brief, conditions were either induced by diluting whole blood (WB) by replacing \textit{via} crystalloids (Sterofundin$^\text{®}$ ISO
Infusionslösung, B. Braun Melsungen AG, Melsungen, Germany) only (hereafter be referred to as haemodilution, HD) or in
combination with lowering the pH value to 6.8 using 2 M HCl and decreasing the temperature from 37 to 32 °C (hereafter be referred to as lethal triad, LT). Constant temperature was realized by continuous storage of samples in an appropriate tempered water bath (Thermolab1070, GFL, Burgwedel, Germany).

**Application of PDMP and EDMP to the simulated HD and LT approaches**

After introducing the conditions of HD and LT, either no MP (untreated controls) or EDMP/PDMP were supplemented in distinct concentrations of 1000 (1k), 10,000 (10k) or 15,000 (15k) MP/µl to the respective condition or whole blood (Figure 1). Exact microparticle quantities required for the simulated approaches had been determined shortly before flow cytometric analysis by using the BD Accuri™ C6 (BD, Heidelberg, Germany). For this purpose, particles were defined by size (0.5 to 0.9 µm) [21] and by typical surface marker originated from the parental cell such as CD42b for platelets and CD144 for endothelial cells, respectively (BD, Heidelberg, Germany). In addition, Annexin V dye was used as marker for the externalisation of phosphatidylserine (PS). After a microparticle incubation of 5 min, samples were processed for subsequent coagulation analysis.

**Extended coagulation analysis**

**Detection of activated platelets**

For measuring platelet activation, cells were fixed according to Cyx III protocol [30] and P-selectin expression was flow cytometric measured by using CD42b and CD62p antibodies (BD. Heidelberg, Germany) and the BD Accuri™ C6 device (BD, Heidelberg, Germany). Results are presented as relative changes of CD42b\(^+\)/CD62p\(^+\) stained platelets referring to the respective unstimulated approach of each experimental group (set as 100%).

**Thrombodynamics™ (TD)**

Differential centrifugation was applied for TD analysis (1.600 x g for 15 minutes followed by a spin of plasma for 5 minutes at 10.000 x g) and platelet-poor plasma (PPP) was immediately shock-frozen in liquid nitrogen and stored at -80 °C until analysis.

For TD analysis, PPP samples of WB and HD were thawed at 37°C and those of LT were thawed accordingly at 32°C in a water bath. The corresponding temperatures for WB, HD and LT were maintained during the TD measurements.

For the determination of spatial clot growth, the TDX kit consisting of reagent I (lyophilized protein of FXIIa inhibitor) and reagent II (CaCl\(_2\)) was used according to the manufacturer's recommendation (HemaCore, Moscow, Russia). Briefly, 120 µl of PPP were supplemented with reagent I and incubated in the device thermostat for 15 min (HemaCore, Moscow, Russia). Subsequently, the PPP samples were treated with reagent II and directly placed into the micro chamber. By inserting immobilized tissue factor, the reaction had been initiated and clot growth and spontaneous clot formation was recorded over 45 min. The following parameters were measured: lag-time (Tlag, min), rate of clot growth (V, µm/min), initial rate of clot growth (Vi, µm/min), clot density (D, a.u.) and clot size (Cs, µm). In addition to these parameters, Cs was measured in five minutes intervals (0-20 min) to estimate the influence of MP on the dynamics of clot growth. In order to statistically record the changed Cs over time, the individual areas under the curve (AUC) of each donor was calculated.

**Statistical analysis**

Statistics and graphical data analyses were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, USA). Non-parametric Friedman test and Dunn's post-hoc test to correct for multiple comparisons were applied to determine significant differences across the collected parameters in the groups of HD and LT.
In a first step, the MP-untreated samples in the groups of WB, HD and LT were compared in pairs as follows: WB vs. HD, WB vs. LT and HD vs. LT. Then, the effects of dose-specific application of PDMP or EDMP were tested in the HD and LT groups by comparing those two. Finally, using a non-parametric Wilcoxon test potential effects between untreated and MP-supplemented samples (C vs. 1k) were analyzed. P-values with a significance level <0.05 were considered statistically significant.

Values with respect to platelet count and TD temporal course of clot growth (Cs) are presented as arithmetical mean and standard deviation.

Results

Demographics of healthy volunteers

Overall, 11 healthy donors were enrolled for analysis. 54.5% of donors were males with a median age of 42 years [IQR 30-52].

Effect of simulated conditions and MP supplementation on platelet count and activation

While the condition of HD led to a marginally decreased platelet count than WB (WB: 2.7 x10^5/µl, [IQR: 0.7x10^5/µl] vs. HD: 2.2 x10^5/µl, [IQR 0.6 x10^5/µl]), the LT caused a significant reduction of cell numbers compared to WB samples (LT: 2.1x10^4, [IQR 2.2x10^3/µl]; p≤0.001; Figure 2 A).

The application of MP resulted in a varying platelet activation depending on the supplemented concentration. Under HD conditions, high PDMP quantities (10k and 15k MP/µl blood) induced an activation of platelets, which was demonstrated by a significant increase of the cell surface marker CD42b^+CD62p^+ expression compared to controls (Figure 2 B). Particularly the administration of 15k PDMP/µl blood further increased platelet activity in this group. Similarly, the highest PDMP concentration (15k MP/µl blood) induced the greatest effect on platelet activation within the LT group compared to the untreated LT controls (Figure 2 B). In contrast to PDMP, EDMP supplementation did not affect platelet activation significantly either group. However, a trend of slight enhancement of activated platelets was observed after supplementation of 10k and 15k EDMP/µl blood (Figure 2 C). Irrespective of the supplemented MP subtype, low concentration (1k of any MP/µl blood) had no effect on platelet activation (Figure 2 B, C).

Kinetics of fibrin clot formation and density after PDMP application

The kinetics of clot formation measured by Tlag, clot growth (V) and the initial rate of clot growth (Vi) remained unaffected in the experimental controls of WB, HD and LT. Only after introduction of low concentrated PDMP (1k PDMP/µl blood) to the whole blood condition a significant change of all clot formation parameters could be observed but remained unaffected under simulated conditions of HD and LT (Table 1). In HD, high PDMP concentrations (10k and 15k PDMP/µl blood) resulted in an enhancement of both growth parameters (Table 1). Under LT conditions, the application of 10k and 15k PDMP/µl blood resulted in an even stronger increase of fibrin growth rate (V) compared to untreated LT controls (Table 1). Furthermore, a tendential but not significant increase of initial clot growth could be observed after PDMP administration in LT samples (Table 1).

In contrast to WB, fibrin polymerization and the associated clot density (D) deteriorated under simulated in vitro HD and LT conditions (Table 1) while a significant reduction compared with untreated WB was detected for LT (Table 1). An improved clot density of fibrin polymerization was achieved by PDMP quantities of 1k MP/µl blood in WB and by high PDMP quantities (10k and 15k MP/µl blood) in HD samples (Table 1). A PDMP stimulating effect on clot density could not be detected for LT (Table 1).
Table 1  Kinetics of fibrin clot formation and density after PDMP application.

| Parameters | PDMP          | WB | HD | LT |
|------------|---------------|----|----|----|
|            | C  | 1k | C  | 1k | 10k | 15k | C  | 1k | 10k | 15k |
| **Tlag [min]** | |     |     |     |     |     |     |     |     |     |
|             | (0.2) |     |     |     |     |     |     |     |     |     |
|             | 0.8* |     |     |     |     |     | 0.9 |     |     |     |
|             | (0.1) |     |     |     |     |     | (0.1) |     |     |     |
| **V [µm/min]** | |     |     |     |     |     |     |     |     |     |
|             | (5.2) |     |     |     |     |     | (6.2) |     |     |     |
|             | 25.5 |     |     |     |     |     | 44.4 |     |     |     |
|             | (3.5) |     |     |     |     |     | (11.4) |     |     |     |
| **Vi [µm/min]** | |     |     |     |     |     |     |     |     |     |
|             | (8.7) |     |     |     |     |     | (6.1) |     |     |     |
|             | 54.2 |     |     |     |     |     | 70.4 |     |     |     |
|             | (9)  |     |     |     |     |     | (5)  |     |     |     |
| **D [a.u]** | |     |     |     |     |     |     |     |     |     |
|             | (5.878) |     |     |     |     |     | (2.951) |     |     |     |
|             | 19.209 |     |     |     |     |     | 14.797|     |     |     |
|             | (6.089) |     |     |     |     |     | (3.447) |     |     |     |
|             | 21.637 |     |     |     |     |     | 14.690 |     |     |     |
|             | (4.876) |     |     |     |     |     | (3.625) |     |     |     |

The following kinetic parameters were determined: Tlag: initial growth rate, V: average clot growth rate and Vi: initial clot growth rate. Clot density was measured as dynamic parameter. Values are presented as median with the corresponding IQR. Statistical significances were marked with plus symbols for differences between unstimulated groups (WB vs. HD and WB vs. LT) or with asterisks for concentration-dependent differences within one experimental group (*, p ≤0.05, **, p ≤0.01, ***, p ≤0.001), respectively. The abbreviation k signifies a thousand.

**Fibrin clot formation following PDMP supplementation - growth and size**

The concentration dependent PDMP application resulted in a significantly increased fibrin clot growth (Cs) over the measured time of 20 min in the WB and HD groups (Figure 3). While the administration of low PDMP concentration 1k PDMP/µl blood caused an enhanced clot growth in WB (Figure 3 A), similar effects had been achieved in HD after supplementing high level of PDMP (10k and 15k PDMP/µl blood, Figure 3 B and Figure 4). The improved fibrin formation in WB and HD was also mirrored in the respective AUCs over a time course of 15 min (Figure 3 D).

Noteworthy, the condition of LT alone led to an increased fibrin growth 15 min after measurement initiation compared to WB controls (Fig. 3 D). However, the supply of PDMP had no further impact except for the addition of 15k PDMP/µl blood at the time point of 10 min (Figure 3 C).

**Spontaneous clotting after EDMP supplementation**

Data collection for the parameters defining fibrin clot growth could not be collected in the EDMP groups due to the early formation of spontaneous clotting originating from EDMP that occurred irrespective of the simulated setting (Figures 5, 6). While no spontaneous clot formation was observed in the control groups of WB and HD, it did occur in LT control conditions (Figure 6).

Even low EDMP concentrations (1k EDMP/µl blood) caused a median spontaneous clot formation in WB (13.4 min, [IQR 21min]), HD (10.1 min, [IQR 14.9 min]) and LT samples (9.5 min, [IQR 9.6 min]) and resulted in unrestricted formation of fibrin clots (Figures 5, 6 A-C). While the supplementation of high EDMP quantities (10k and 15k EDMP/µl blood) initiated spontaneous clotting in HD samples after one minute (1.55 min, [IQR 3.4 min] vs. 1.7 min, [IQR 2.7 min]; Figure 6 B), a firm clot filling in the whole measuring cuvette was observed after two minutes (Figure 5). Within the LT condition a similar effect could be observed when supplementing high EDMP concentrations (10k EDMP/µl blood: 1.35 min, [IQR 1.6 min]; 15k EDMP/µl blood: 1.75 min, [IQR 1.45 min]; Figure 6 C and Figure 5). In all simulated approaches, spontaneous clot formation led to the expansion of clot propagation up to a firm clot in the measuring cuvette, which originated from the spontaneous clotting spots.

**Discussion**
The present study aimed to elucidate the role of PDMP and EDMP on functional coagulation under *in vitro* simulated conditions. In line with previous findings, the applied experimental settings of HD and LT in this study were appropriate models for standardized *in vitro* simulations of an dilutional coagulopathy that may occur after trauma [28,29].

With this study we demonstrated for the first time a divergent mechanism of action on coagulation originating from MP with different parental cells. Special emphasis was given to the analysis of selected MP on blood coagulation with a particular focus on PDMP and EDMP. Both MP subtypes had been reported to be released in high quantities and a specific distribution pattern in patients who sustained major traumatic injuries. But whether these particles contribute to the pathomechanism of TIC or are involved in a regulation pathway after trauma is still a matter of research [19,25,31,32]. Within our study design, supplemented PDMP mainly affected circulating platelets by inducing their activation, which was measured by enhanced P-selectin expression on platelet membranes (CD42b⁺/CD62p⁺), particularly, but not exclusively, in HD conditions. It is conceivable that an increased platelet activation likely compensates for reduced platelet counts in HD and LT conditions potentially via the ligand-receptor interaction between the P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) forming a PSGL-1/P-selectin complex that leads to exponential cell activation [33–36]. In trauma patients it has been demonstrated that increased cell activation is associated with a promoted phosphatidylserine (PS) exposure resulting in procoagulant platelet formation and increased procoagulant activity of PS positive MPs [18,19,37]. In addition, this activation lead to a release of coagulation-promoting components from the platelet storage granules [38]. Furthermore, not only platelets expose PS on their membrane, but also *in vitro* generated PDMP as determined in this study by flow cytometric measurements. It is well known that PS on platelet surfaces cause an assembly of coagulation factors promoting functional clotting [39,40]. Related to our findings Lipets *et al.* also revealed that *in vitro* generated PDMP contribute to an enhanced coagulation propagation by expressing PS on their cell membrane [41].

PDMP together with activated platelets could result in a surface expansion for clotting factors, which then would explain the locally increased formation of thrombin and fibrin polymerization under high PDMP treatment mainly in HD samples.

In contrast to PDMP, *in vitro* generated EDMP affected the impaired coagulation of HD and LT unexpectedly not by activating platelets, but by inducing spontaneous clotting originating from the particles itself. Therefore, the expected EDMP-mediated platelet activation via membrane P-selectin seems to play a secondary role in coagulation stimulation, which is likely due to a lower P-selectin exposure present on *in vitro* generated EDMP, which seem to be insufficient to activate platelets in a way PDMP do.

Furthermore, we observed a concentration-dependent mode of action for both PDMP and EDMP as both particles revealed a stronger effect in higher concentrations (10k or 15k MP/µl blood). For EDMP, the concentration effect mainly accelerated spontaneous clotting in HD (<5 min) and LT (<3 min) conditions as the process itself was not concentration-dependent. Already low EDMP concentrations (1k MP/µl blood) led to spontaneous fibrin formation. An underlying mechanism by which EDMP cause spontaneous clotting could be based on the presence of tissue factor (TF) on EDMP, which has been shown to be released after targeted stimulation from human endothelial cells *in vitro* [42–44]. TF in turn mediates a coagulation initiation via the coagulation factor VII/TF-dependent extrinsic pathway and likely results in spontaneous fibrin formation [45,46]. The earlier formation of spontaneous fibrin clots following EDMP administration in LT in comparison to HD potentially resulted from the combination of both, the TF-bearing EDMP and the acidosis-related increased basal platelet activation under LT conditions.

As recently published by our group, there is a positive correlation between injury severity and MP concentration after trauma likely reaching high quantities inducing procoagulant effects [25]. Our *in vitro* experimental setup indicates that under LT conditions MP promote procoagulant function by different pathways based on their cellular origin. Therefore, at first sight, our data seems to be contrary to recent clinical observations of platelet dysfunction in TIC-related patients showing reduced platelet activity levels after trauma [16,17]. These clinical data reflect the final common path of the multicausal pathophysiology of TIC resulting in the hypocoagulative state after trauma. The results from our experimental model
should only be carefully extrapolated to a clinical picture but describes the procoagulant potential. It remains to be conclusively clarified and requires further investigation whether and how MP are involved in the pathophysiology of TIC after major trauma *in vivo* and whether those high quantities as used in this study can, in fact, be physiologically achieved in a clinical setting.

**Conclusion**

The present study revealed a procoagulant role of PDMP and EDMP under circumstances of a dilutional coagulopathy as it occurs after major traumatic injury. Particularly, high concentrations of both MP types showed effects on the coagulation process. A divergent mechanism of action indicate that these MP subtypes have diverse function in stimulating coagulation under the investigated conditions of HD and LT. Likely due to the platelet-mediated activation (increased P-selectin expression) and the presence of PS lipids on PDMP membranes, clotting factor assembly and thus clot formation could be promoted on PDMP surfaces. In association with high EDMP quantities, an improved coagulation with formation of spontaneous fibrin clots was determined, which could presumably be induced via the TF/FVIIa-dependent extrinsic pathway of coagulation.

**Limitations**

It would have been desirable to perform the analysis with isolated MP subtypes from real trauma patients. But since high MP quantities would have been required for this study and the extraction capacity from patient's blood is limited, we relied on *in vitro* synthesized MP. There is a limitation in interpreting the results as *in vitro* LPS-stimulated MP might have a higher procoagulant activity compared to *in vivo* MP [47]. However, during the validation process of our assays, we compared the activity of *in vitro* generated MP to that of *in vivo* MP and did not see any difference.

Additionally, we are aware of the fact that the current *in vitro* study reflects the pathophysiological mechanism present in trauma patients only in part. For this reason, findings should cautiously be transferred to a clinical setting.

**Abbreviations**

ATC: Acute traumatic coagulopathy, C\(_S\): Clot size, CMMC: Cologne-Merheim Medical Centre, EDMP: D: Clot density, Endothelial-derived microparticles, HD: Haemodilution, IC: Iatrogenic coagulopathy, ITM: Institute of Transfusion Medicine, LPS: Lipopolysaccharide LT: Lethal triad, MP: Microparticles; PDMP: Platelet-derived microparticles, PPP: platelet-poor plasma; PS: Phosphatidylserine, PSGL-1: P-Selectin gylcoprotein ligand-1, TD: Thrombodynamics, TIC: Trauma-induced coagulopathy; Tlag: lag-time, TF: Tissue factor, Tsp: Time of spontaneous clot formation, V: Rate of clot growth, Vi: initial rate of clot growth, WB: Whole blood

**Declarations**

**Ethics approval and consent to participate**

Study approval was given by the ethical committee of Witten/Herdecke University (#182/2016).

**Consent for publication**

All volunteers (blood donors) gave their written and informed consent for participating in this study.

**Acknowledgement**

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Availability of data and materials

All data that are relevant for the study are included in this published article. Further datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

MC, NS and MM designed the study. JB, NS collected, analyzed and interpreted the data, wrote the manuscript, which has been critically reviewed by MM, MC, BS and UB. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Figures
Figure 3

Dynamic fibrin increases over time measured by clot size in whole blood (WB), haemodilution (HD) and lethal triad (LT) after PDMP supplementation (A-C). Values are represented as mean ± standard deviation. The respective AUCs at 15 minutes (D) following measurement initiation are depicted as Tukey-boxes. Significances are indicated with the following symbol for the following comparisons: diamond - controls vs. 1k PDMP, cross – controls vs. 10k PDMP and hash - controls and 15k PDMP. Statistical significances regarding AUCs were marked with plus symbols for differences between unstimulated groups (WB vs. HD and WB vs. LT) or with asterisks for concentration-dependent differences within one experimental group. Irrespective the comparison one symbol indicates $p \leq 0.05$, two symbols $p \leq 0.01$ and three symbols $p \leq 0.001$. The abbreviation k signifies a thousand.