COLLECTION, PRODUCTION AND STORAGE OF BLOOD COMPONENTS

*Original article*

# The effects of pathogen reduction technology on apheresis platelet concentrates stored in PAS

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**Background** - The impact of pathogen reduction technology (PRT) such as Mirasol, and the effect of platelet additive solutions (PAS) on the activity and hemostatic profile of transfused apheresis platelets remain largely unknown. The aim of this study was to assess the *in vitro* hemostatic and metabolic profile of Mirasol treated platelets in PAS during a 7-day storage period.

**Material and methods** - Ten split bags containing apheresis platelets stored in PAS were split into two groups; control platelets (No.=10 units) and PRT-treated platelets (No.=10 units). *In vitro* evaluation of the platelet components was performed on the 1st, 3rd, 5th, and 7th days of the storage period. Several metabolic parameters including pH, glucose, and lactate levels were evaluated, while assessment of their hemostatic capacity was performed using light transmission aggregometry (LTA) and viscoelastic studies such as rotational thromboelastometry (ROTEM) and thromboelastography (TEG). Last, Annexin V levels were measured though flow cytometry for evaluation of platelet activation.

**Results** - Clot strength, as reflected by the maximum clot firmness (MCF) and the maximum amplitude (MA) parameters of the viscoelastic studies was significantly decreased in the PRT-treated platelets compared to the control platelets ($p<0.05$). Clot strength based on MCF and MA values was also found to be decreasing over storage time in PRT-treated platelets ($p<0.001$), while this was not evident in control platelets. Moreover, the comparison between pH, glucose, and lactate levels were indicative of increased metabolic activity in PRT-treated platelets compared to control platelets ($p<0.001$). Last, Annexin-V was significantly higher in PRT-treated platelets compared to control platelets on the 7th day of the storage period ($p<0.001$).

**Discussion** - The results of this study indicate that increased PSL induced by PRT treatment leads to a decreased *in vitro* platelet hemostatic efficacy and increased metabolic activity. However, the clinical impact of these alterations needs further investigation.

**Keywords:** *apheresis platelets, TEG, ROTEM, pathogen reduction technology, platelet storage efficacy.*

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## INTRODUCTION

Blood bank primary goals include adequate supply of the requested blood products while ensuring the highest level of safety and quality of the available transfusion products. Safety is associated with prevention of pathogen transmission, whereas quality assessment involves optimization of the storage conditions in order to mitigate the effect of any degradation process due to storage time on the therapeutic efficacy of the transfused products. In order to adhere to the aforementioned safety standards, pathogen reduction technology (PRT) systems have been developed, while an increasingly popular strategy to mitigate platelet storage lesions (PSL) is the use of platelet additive solution (PAS) in platelet suspensions[1]. PAS is a balanced electrolyte solution with standardized composition that can be steam-sterilized and potentially reduce antibodies associated with transfusion related acute lung injury (TRALI), while PRT systems use UV radiation for photochemical treatment to prevent replication of pathogens in platelet concentrates (PCs)[2]. These systems modify afflicted DNA molecules and change their molecular structure, affecting both pathogens and white blood cells contained in apheresis platelets[3-6].

The two most commonly used PRT systems nowadays include the Intercept and Mirasol systems. Intercept uses a combination of amotosalen and UVA (Cerus Corporation, Concord, CA, USA) to prevent pathogen replication, while residual amotosalen at the end of this procedure is removed to avoid any potential toxicity. The second system, Mirasol (Mirasol PRT, Terumo BCT, Lakewood, CO, USA), utilizes riboflavin (vitamin B2) and broad spectrum UVB light to achieve pathogen reduction. As opposed to Intercept, riboflavin does not need to be removed from the final product at the end of the precedure[7]. Recently, a third PRT system has been developed, the THERAFLEX UV-Platelets (MacoPharma, Mouvaux, France), which uses short-wave ultraviolet light UVC applied to PCs under agitation without any photosensitizer[7,8]. However, this system has not been widely used so far.

PRTs provide an extra safety measure against emerging blood-borne infectious diseases, while they also result in prolongation of PCs shelf life up to 7 days. There is a great body of evidence supporting the efficacy of PRTs in mitigating transmission of various pathogens such as West Nile virus and coronaviruses[9-11]. Furthermore, Mirasol was recently shown to be effective in reducing SARS-CoV-2 in plasma, platelets, and whole blood products[12,13]. The impact of PRT on PSL has been an issue of great interest over the past years. PSLs include elevated proteolysis, changes in morphology to spherical shape, pH decline due to upregulated metabolism, and induction of the apoptotic process along with increased expression of surface receptors[14,15]. Although these degradation changes occur naturally, they seem to be enhanced in PRT- treated platelets. The impact of PRT treatment on the metabolic profile and hemostatic efficacy of treated platelets suspended in PAS remains a subject of debate. The main purpose of this study was to perform an *in vitro* investigation of the metabolic and hemostatic profile of PRT-treated platelets, through comparison of the functional and coagulation properties of PRT-treated and untreated platelets.

## MATERIAL AND METHODS

### In vitro collection

Ten split double-dose apheresis PCs stored in a mix of 65% T-PAS+, a 3rd generation PAS-E, and 35% plasma were produced. T-PAS+ contains magnesium chloride hexahydrate, potassium chloride, sodium dihydrogen phosphate dihydrate, sodium citrate dihydrate, sodium chloride, sodium acetate trihydrate, disodium hydrogen phosphate dodecahydrate. Platelet collection was performed using a Trima collection device (Trima, Accel Terumo BCT, Lakewood, CO, USA). Per manufacturer's instructions a protocol was set up, and approximately $6.5\times10^{11}$ platelets were collected in 510 mL of storage solution in each apheresis bag. The collected platelets were suspended in approximately 340 mL solution (65% PAS and 35% plasma), along with 35 mL platelet poor plasma (PPP) in a separate bag to be used for any test applicable. Apheresis platelets were kept undisturbed for one hour at 22-24°C to allow disaggregation of any platelet aggregates, after which they were agitated for one hour before being divided into two platelet aliquots of roughly the same size. One PC bag was marked as a control unit (C) and the other one was marked as mirasol treated unit (M) and transferred along with a riboflavin kit in low light conditions, in an illumination bag. The M aliquot was UV-treated in a Mirasol device according to the manufacturer's instructions, being linearly agitated at 120 cpm and 37°C. The target energy to be delivered was 6.24 J/mL.





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Both platelet aliquots were stored in the same linear agitator at 20-24°C for seven days. Samples were collected under aseptic conditions on the 1st, 3rd, 5th and 7th day. All performed tests were completed within 4 hours from the collection time.

The following parameters were estimated: platelet count, metabolic markers (pH, $pO_2$, $pCO_2$, lactate, glucose and lactate dehydrogenase [LDH]), platelet aggregation through Light Transmission Aggregometry (LTA), platelet activation (annexin-V expression) through flow cytometry, and viscoelastic properties through Thromboelastography (TEG) and Rotational Thromboelastometry (ROTEM).

The study was approved by the "Attikon" General University Hospital's Institutional Review Board (30/07/2021). Prior to any platelet apheresis procedure, the donor was informed about the study protocol and all recruited platelet donors gave a written informed consent.

### Blood gas and metabolism assays

Platelet count measurements were performed on a Sysmex XE-2100 analyzer (Roche, Lincolnshire, IL, USA). Blood gas and pH analysis was conducted using GEM Premier 5000 blood gas analyzer (Instrumentation Laboratory, Bedford, MA, United States), while lactate and glucose levels were estimated by GEM Premier 5000 analyzer (Instrumentation Laboratory, Bedford, MA, USA). Last, LDH levels were measured by Cobas® 8000 (Roche Diagnostics Ltd, Rotkreuz, Switzerland) analyzer.

### Light transmission aggregometry (LTA)

The platelet count for each control and treated sample was adjusted between $200\times10^9$/L and $300\times10^9$/L using donor-PPP. LTA measurements were performed by Biodata-PAP-4 aggregometer (Bio-Data Corporation, Horsham, PA, USA). For the measurements to be carried out one agonist was used, ADP 2.0×10-5 M (Bio-Data Corporation). Tests were performed based on established set of techniques per previous instructions[16]. A sample of 450 μL platelet rich plasma (PRP) was placed in an appropriate translucent cuvette and was incubated at 37°C for 3 mins. Following that 3-minute period, 50 μL of agonist were added and the aggregation procedure was allowed to proceed for 10 minutes.

### ROTEM

The viscoelastic properties of platelet samples were evaluated using a ROTEM analyzer (Tem Innovations GmbH, Munich, Germany). In order to obtain measurements, platelet samples were diluted at a ratio of 1:5 with poor platelet donor plasma which was ultracentrifuged and frozen at −40°C beforehand, in aliquots that were discarded after a single thawing[17]. All ROTEM analyses were performed according to the manufacturers' whole blood instructions within 2h from sample collection[17]. The EXTEM and FIBTEM assays were performed. Specifically, for the EXTEM assay a recombinant tissue factor was used to activate the extrinsic coagulation pathway, while for the FIBTEM assay cytochalasin D was additionally added to inhibit platelet contribution to coagulation. The following parameters were evaluated: clotting time (CT in sec), clot formation time (CFT in sec), clot amplitude recorded at 10 minutes (A10 in mm), and maximum clot firmness (MCF in mm). Last, the calculate maximum clot elasticity was estimated based on the following formula: $MCE=(MCF\times100)/(100-MCF)$.

### TEG

Thromboelastography is another laboratory method that estimates global viscoelastic properties under low shear stress. This analysis was carried out with a TEG 5000 analyzer (Haemoscope Corporation, Niles, IL, USA). Platelet samples were diluted at a ratio of 1:5 with poor platelet donor plasma, which was ultracentrifuged and frozen at −40°C beforehand, in aliquots that were discarded after a single thawing. Samples were run according to the manufacturers' whole blood instructions, within 2h from sample collection[18]. Rapid-TEG (r-TEG) was performed utilizing tissue factor instead of the kaolin-cephalin reagent to activate blood coagulation. Due to the nature and number of the involved coagulation factors, these tests can be performed faster than conventional TEG. The following TEG parameters were evaluated: reaction time (R-time), kinetic time (K-time), alpha angle (α-angle), and maximum amplitude (MA).

### Flow cytometry

To identify receptors indicating platelet activation, flow cytometry was performed using a FACSCanto II cytometer (BD Biosciences, San Jose, CA, USA) with multiple color lasers. Specifically, annexin-V bound to the exposed phosphatidyl serine on the surface of platelets was measured during the 7-day storage period. Samples were incubated for 15 mins at room temperature with Annexin-V with PE coloring and the platelet gating marker

**Table I** - *Metabolic parameters in Mirasol-treated and control platelets*

| Parameter/day | Median (IQR) | | p-value |
|---|---|---|---|
| | Mirasol | Control | |
| **Volume, 1** | 264.5 (255-310) | 220.5 (209-230) | **<0.001** |
| **Volume, 3** | 272.5 (254-298) | 218 (188-222) | **<0.001** |
| **Volume, 5** | 254.5 (238-286) | 196.5 (174-205) | **<0.001** |
| **Volume, 7** | 240 (222-272) | 179.5 (160-188) | **<0.001** |
| **p-value** | 0.06 | **0.002** | |
| **Concentration ($10^3$/uL), 1** | 175 (156-198) | 213 (190-232) | **0.034** |
| **Concentration ($10^3$/uL), 3** | 201.5 (164-226) | 223.5 (191-251) | 0.18 |
| **Concentration ($10^3$/uL), 5** | 205 (159-208) | 209 (191-241) | 0.32 |
| **Concentration ($10^3$/uL), 7** | 180.5 (132-202) | 198.5 (166-230) | 0.28 |
| **p-value** | 0.41 | 0.68 | |
| **pH, 1** | 7.15 (7.11-7.17) | 7.17 (7.12-7.19) | 0.13 |
| **pH, 3** | 6.91 (6.82-6.99) | 7.24 (7.22-7.33) | **<0.001** |
| **pH, 5** | 6.82 (6.80-6.87) | 7.25 (7.21-7.27) | **0.001** |
| **pH, 7** | 6.88 (6.83-6.91) | 7.20 (7.16-7.26) | **<0.001** |
| **p-value** | **<0.001** | **0.018** | |
| **$pO_2$ (mmHg), 1** | 62 (46-80) | 53.5 (47-86) | 0.96 |
| **$pO_2$ (mmHg), 3** | 98.5 (82-109) | 83.5 (53-108) | 0.36 |
| **$pO_2$ (mmHg), 5** | 108 (103-123) | 77.5 (58-93.5) | **0.005** |
| **$pO_2$ (mmHg), 7** | 122 (114-132) | 79.5 (60-85) | **0.003** |
| **p-value** | **0.003** | 0.27 | |
| **$pCO_2$ (mmHg), 1** | 15.5 (14-16) | 18 (15-20) | 0.06 |
| **$pCO_2$ (mmHg), 3** | 12.5 (11-16) | 12 (9-13) | 0.20 |
| **$pCO_2$ (mmHg), 5** | 10.5 (9-13) | 11 (10-11) | 0.90 |
| **$pCO_2$ (mmHg), 7** | 8 (5-8) | 10.5 (9-12) | **0.007** |
| **p-value** | **<0.001** | **<0.001** | |
| **Glucose (mg/dL), 1** | 86 (78-94) | 94 (91-106) | **0.030** |
| **Glucose (mg/dL), 3** | 23.5 (16-36) | 66 (58-83) | **<0.001** |
| **Glucose (mg/dL), 5** | 0 (0-0) | 43.5 (32-51) | **<0.001** |
| **Glucose (mg/dL), 7** | 0 (0-0) | 19 (6-30) | **<0.001** |
| **p-value** | **<0.001** | **<0.001** | |
| **Lactate (mmol/L), 1** | 1.4 (1.3-1.8) | 1.5 (1.4-1.9) | 0.54 |
| **Lactate (mmol/L), 3** | 9.1 (8.2-10.4) | 5.2 (4.4-5.7) | **<0.001** |
| **Lactate (mmol/L), 5** | 12.4 (11.6-13.0) | 7.5 (7.1-8.6) | **0.001** |
| **Lactate (mmol/L), 7** | 12.4 (11.4-13.6) | 11 (9-12.1) | **0.037** |
| **p-value** | **<0.001** | **<0.001** | |
| **LDH (IU/L), 1** | 38 (13-61) | 46.5 (23-72) | 0.42 |
| **LDH (IU/L), 3** | 55 (36-78) | 81 (65-143) | **0.046** |
| **LDH (IU/L), 5** | 86.5 (73-218) | 78.5 (69-98) | 0.24 |
| **LDH (IU/L), 7** | 139 (79-543) | 93 (81-148) | 0.40 |
| **p-value** | **<0.001** | **0.037** | |

p-values in bold stand for statistically significant results (p<0.05). IQR: interquartile range; LDH: lactate dehydrogenase.

CD41a-PECy5. Afterwards, they were suspended in Buffer solution and flow cytometry analysis was performed and processed using the BD FACSDiva software[19].

### Statistical analysis

The evaluated variables were presented using median values and interquartile ranges (IQR). The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare the variables between the Mirasol-treated and the control PCs, while the Kruskal-Wallis equality-of-populations rank test was used to compare the variables among the different days (day 1, 3, 5 and 7). Correlations between the laboratory parameters were evaluated using the Spearman rank correlation coefficient (Spearman's rho categories of correlation: r<0.20, very weak correlation; 0.21<r<0.40 weak correlation; 0.41<r<0.60, moderate correlation; 0.61<r<0.80, strong correlation; r>0.81 very strong correlation). Statistical significance was set at p <0.05 for all tests, while the Stata software (Stata Corp., College Station, TX, USA) was used for the statistical analysis.

## RESULTS

The results of the metabolic and hemostatic parameters for the Mirasol-treated platelets and untreated platelets are presented in **Table I** and **Table II** respectively. Regarding the metabolic markers, glucose consumption and lactate production were significantly increased over

**Table II** - *Hemostatic parameters in Mirasol-treated and control platelets*

| Parameter/day | Median (IQR) | | p-value |
|---|---|---|---|
| | Mirasol | Control | |
| **LTA ADP (%),** 1 | 30 (21-57) | 41.5 (9-61) | 0.96 |
| **LTA ADP (%), 3** | 17.5 (7-28) | 3.5 (1-9) | **0.017** |
| **LTA ADP (%), 5** | 6.5 (0-16) | 4 (2-11) | 0.96 |
| **LTA ADP (%), 7** | 1.5 (0-8) | 6 (2-15) | 0.24 |
| **p-value** | **<0.001** | **0.002** | |
| **Annexin-V, 1** | 2.6 (1.6-4) | 2.6 (1.9-5) | 0.96 |
| **Annexin-V, 3** | 6.1 (4-8.3) | 4.6 (3.5-5.8) | 0.27 |
| **Annexin-V, 5** | 7.8 (4.3-12.4) | 6.4 (3.6-9.3) | 0.48 |
| **Annexin-V, 7** | 37.2 (29.1-48.6) | 6.2 (4.3-7) | **<0.001** |
| **p-value** | **<0.001** | 0.13 | |
| **CT EXTEM (sec), 1** | 82.5 (69-95) | 79.5 (71-88) | 0.73 |
| **CT EXTEM (sec), 3** | 76.5 (65-89) | 67.5 (61-79) | 0.22 |
| **CT EXTEM (sec), 5** | 70.5 (69-73) | 69 (66-74) | 0.76 |
| **CT EXTEM (sec), 7** | 72.5 (65-81) | 70.5 (68-73) | 0.62 |
| **A10 EXTEM (mm), 1** | 56.5 (49-57) | 59 (54-63) | 0.30 |
| **A10 EXTEM (mm), 3** | 33.5 (29-43) | 54 (44-58) | **0.012** |
| **A10 EXTEM (mm), 5** | 23 (19-27) | 51.5 (42-55) | **0.001** |
| **A10 EXTEM (mm), 7** | 22 (19-29) | 49.5 (45-56) | **0.001** |
| **p-value** | **<0.001** | 0.17 | |
| **A10 FIBTEM (mm), 3** | 19 (17-22) | 20.5 (17-23) | 0.44 |
| **A10 FIBTEM (mm), 5** | 20 (17-21) | 21 (20-22) | 0.10 |
| **A10 FIBTEM (mm), 7** | 20 (17-24) | 20.5 (18-24.5) | 0.68 |
| **p-value** | 0.86 | 0.71 | |
| **CFT EXTEM (sec), 1** | 55.5 (46-82) | 48.5 (40-65) | 0.40 |
| **CFT EXTEM (sec), 3** | 66 (58-94) | 54 (46-86) | 0.09 |
| **CFT EXTEM (sec), 5** | 109 (86-130) | 61 (51-91) | **0.005** |
| **CFT EXTEM (sec), 7** | 147 (88-543) | 54 (51-73) | **<0.001** |
| **p-value** | **<0.001** | 0.46 | |
| **MCF EXTEM (mm), 1** | 60 (52-61) | 61 (57-67) | 0.40 |
| **MCF EXTEM (mm), 3** | 37.5 (30-43) | 54.5 (45-59) | **0.009** |
| **MCF EXTEM (mm), 5** | 24.5 (19-32) | 51.5 (43-56) | **<0.001** |
| **MCF EXTEM (mm), 7** | 24 (19-30) | 49.5 (45-57) | **0.001** |
| **p-value** | **<0.001** | 0.058 | |
| **MCF FIBTEM (mm), 3** | 21 (19-24) | 22.5 (20-25) | 0.36 |
| **MCF FIBTEM (mm), 5** | 22 (19-25) | 23 (23-25) | 0.38 |
| **MCF FIBTEM (mm), 7** | 22 (20-27) | 23 (21-26.5) | 0.68 |
| **MCE EXTEM, 3** | 60.5 (43-75) | 121 (81-143) | **0.009** |
| **MCE EXTEM, 5** | 30.5 (24-41) | 106 (74-125) | **<0.001** |
| **MCE EXTEM, 7** | 31.5 (24-43) | 97 (81-130) | **0.001** |
| **p-value** | **<0.001** | 0.059 | |
| **MA TEG (mm), 1** | 75.3 (73.2-76.7) | 77.5 (76.6-80.3) | 0.10 |
| **MA TEG (mm), 3** | 72.4 (70.0-75.1) | 76.8 (75.5-78.1) | **0.010** |
| **MA TEG (mm), 5** | 69.4 (65.9-70.8) | 76.7 (75.5-78.9) | **0.001** |
| **MA TEG (mm), 7** | 60.3 (52.0-63.2) | 76.5 (75.0-81.0) | **<0.001** |
| **p-value** | **<0.001** | 0.90 | |
| **R TEG (min), 1** | 0.7 (0.6-0.8) | 0.7 (0.6-0.7) | 0.72 |
| **R TEG (min), 3** | 0.6 (0.5-0.7) | 0.7 (0.5-0.7) | 0.71 |
| **R TEG (min), 5** | 0.5 (0.5-0.8) | 0.6 (0.6-0.7) | 0.34 |
| **R TEG (min), 7** | 0.6 (0.5-0.7) | 0.7 (0.6-0.8) | 0.35 |
| **p-value** | 0.68 | 0.73 | |
| **K TEG (min), 1** | 0.8 (0.8-0.8) | 0.8 (0.8-0.8) | 0.95 |
| **K TEG (min), 3** | 0.8 (0.8-0.8) | 0.8 (0.8-0.8) | 0.31 |
| **K TEG (min), 5** | 0.8 (0.8-0.8) | 0.8 (0.8-0.8) | 0.14 |
| **K TEG (min), 7** | 0.9 (0.8-1.2) | 0.8 (0.8-0.8) | **0.018** |
| **p-value** | **0.006** | 0.39 | |

p-values in bold stand for statistically significant results (p<0.05). IQR: interquartile range; LTA: light transmission aggregometry; EPI: epinephrine; ADP: adenosine diphosphate; CT: clotting time; CFT: clot formation time; A10: amplitude 10 min after CT; MCF: maximum clot firmness; LI60: lysis index at 60 minutes; MCE: maximum clot elasticity; TEG: thromboelastography; MA: maximum amplitude; LY60: lysis value at 60 minutes.

time (p<0.001) in both treated and untreated platelets, while glucose consumption and lactate production were higher in Mirasol-treated platelets compared to control ones, starting from the 3rd day of the storage period. Moreover, glucose reserves were depleted from the 5th day of the storage period in Mirasol-treated platelets, while control samples retained a far better glucose reserve (p<0.001) and lower lactate levels. The mean pH levels of the treated platelets were significantly lower (p<0.001) on the 3rd and 7th day compared to those of the control samples

**Table III** - *Spearman correlation coefficients in control platelets*

| | Volume | Concentration | pH | $pO_2$ | $pCO_2$ | Glucose | Lactate | LDH | $HCO_3$ | LTA ADP | Ann V binding | MA TEG | K TEG | MCF EXTEM | MCE EXTEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Volume** | 1 | | | | | | | | | | | | | | |
| **Concentration** | 0.30 | **1** | | | | | | | | | | | | | |
| **pH** | −0.23 | −0.07 | 1 | | | | | | | | | | | | |
| **$pO_2$** | **−0.21** | **−0.41** | **−0.54** | 1 | | | | | | | | | | | |
| **$pCO_2$** | **0.54** | **0.38** | **−0.57** | **−0.33** | **1** | | | | | | | | | | |
| **Glucose** | **0.53** | 0.10 | 0.02 | −0.29 | **0.61** | **1** | | | | | | | | | |
| **Lactate** | **−0.61** | −0.07 | 0.04 | 0.17 | **−0.64** | **−0.93** | 1 | | | | | | | | |
| **LDH** | 0.01 | 0.30 | 0.05 | −0.09 | −0.18 | **−0.32** | **0.40** | **1** | | | | | | | |
| **$HCO_3$** | 0.25 | 0.27 | −0.22 | −0.24 | **0.77** | **0.75** | **−0.75** | −0.28 | **1** | | | | | | |
| **LTA ADP** | 0.01 | −0.22 | −0.05 | −0.16 | 0.24 | **0.37** | −0.29 | −0.21 | 0.20 | **1** | | | | | |
| **LTA EPI** | −0.24 | **−0.40** | 0.10 | −0.05 | −0.10 | 0.07 | 0.01 | **−0.33** | −0.22 | **0.66** | | | | | |
| **Ann V binding** | −0.01 | 0.13 | −0.14 | 0.05 | −0.18 | −0.31 | **0.45** | **0.40** | −0.28 | −0.20 | **1** | | | | |
| **MA TEG** | 0.25 | **0.49** | −0.29 | **−0.51** | **0.36** | 0.03 | −0.06 | 0.30 | 0.11 | −0.21 | 0.26 | **1** | | | |
| **K TEG** | −0.18 | −0.22 | −0.07 | 0.27 | −0.27 | −0.26 | 0.27 | 0.11 | −0.16 | −0.24 | 0.24 | −0.27 | **1** | | |
| **MCF EXTEM** | **0.54** | **0.59** | −0.18 | **−0.49** | **0.51** | 0.44 | **−0.41** | 0.06 | **0.41** | −0.05 | 0.10 | **0.49** | −0.27 | 1 | |
| **MCE EXTEM** | 0.55 | **0.59** | −0.17 | **−0.50** | **0.50** | 0.44 | **−0.42** | 0.07 | **0.41** | −0.05 | 0.01 | **0.49** | −0.27 | 0.99 | 1 |

In bold statistically significant results. LDH: lactate dehydrogenase; LTA: light transmission aggregometry; ADP: adenosine diphosphate; EPI: epinephrine; MA: maximum amplitude; MCF: maximum clot firmness; MCE: maximum clot elasticity.

**Table IV** - *Spearman correlation coefficients in Mirasol-treated platelets*

| | Volume | Concentration | pH | $pO_2$ | $pCO_2$ | Glucose | Lactate | LDH | $HCO_3$ | LTA ADP | Ann V binding | MA TEG | K TEG | MCF EXTEM | MCE EXTEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Volume** | 1 | | | | | | | | | | | | | | |
| **Concentration** | −0.23 | **1** | | | | | | | | | | | | | |
| **pH** | 0.30 | −0.13 | 1 | | | | | | | | | | | | |
| **pO2** | **−0.24** | −0.19 | **−0.54** | 1 | | | | | | | | | | | |
| **pCO2** | **0.48** | 0.14 | **0.35** | **−0.61** | 1 | | | | | | | | | | |
| **Glucose** | **0.32** | −0.12 | **0.79** | **−0.65** | **0.69** | 1 | | | | | | | | | |
| **Lactate** | **−0.40** | 0.12 | **−0.83** | **0.53** | **−0.61** | **−0.90** | 1 | | | | | | | | |
| **LDH** | **−0.60** | 0.11 | **−0.61** | **0.54** | **−0.68** | **−0.70** | **0.61** | 1 | | | | | | | |
| **HCO3** | 0.25 | −0.12 | **0.64** | −0.24 | **0.68** | **0.73** | **−0.87** | −0.27 | 1 | | | | | | |
| **LTA ADP** | **0.50** | −0.04 | **0.42** | −0.59 | **0.70** | **0.64** | **−0.49** | **−0.68** | 0.48 | 1 | | | | | |
| **LTA EPI** | **0.44** | −0.20 | 0.22 | −0.36 | **0.47** | **0.42** | **−0.33** | **−0.56** | 0.28 | **0.72** | | | | | |
| **Ann V binding** | **−0.40** | −0.16 | **−0.50** | 0.59 | **−0.79** | **−0.72** | **0.70** | **0.59** | **−0.49** | **−0.53** | 1 | | | | |
| **MA TEG** | **0.31** | 0.22 | **0.40** | −0.74 | **0.72** | **0.64** | **−0.54** | **−0.52** | 0.37 | **0.69** | **−0.58** | 1 | | | |
| **K TEG** | −0.01 | −0.16 | −0.11 | 0.53 | **−0.49** | **−0.39** | 0.29 | 0.31 | −0.33 | **−0.36** | 0.46 | **−0.61** | 1 | | |
| **MCF EXTEM** | 0.12 | 0.14 | **0.71** | −0.73 | **0.68** | **0.80** | **−0.75** | **−0.55** | 0.64 | **0.60** | −0.64 | **0.75** | **−0.62** | 1 | |
| **MCE EXTEM** | 0.13 | 0.16 | **0.72** | −0.74 | **0.69** | **0.81** | **−0.74** | **−0.57** | 0.65 | **0.63** | −0.67 | **0.75** | **−0.61** | **0.97** | 1 |

In bold statistically significant results. LDH: lactate dehydrogenase; LTA: light transmission aggregometry; ADP: adenosine diphosphate; EPI: epinephrine; MA: maximum amplitude; MCF: maximum clot firmness; MCE: maximum clot elasticity.

but remained within acceptable limits during the 7-day storage period. Last, LDH values showed a significant increase in both groups during the 7-day storage period (p<0.001 and p=0.037 respectively), with no in-between significant differences.

Annexin-V showed a significant increase during storage time in treated platelets (p<0.001), while Annexin-V in treated platelets was significantly higher than that of the untreated platelets on the 7th day (p<0.001). Regarding the evaluation of the hemostatic capacity, aggregation with ADP agonist significantly decreased over storage time in both treated and untreated platelets, although the obtained values were comparable for both groups. Regarding the viscoelastic methods, the maximum clot firmness (MCF) in the EXTEM assay of the ROTEM analysis was significantly lower in Mirasol-treated platelets on the 3rd, 5th and 7th day compared to those of the untreated platelets (p=0.009, p<0.001, p=0.001, respectively). In addition, treated platelets had a significant decrease in EXTEM A10, MCE and CFT values over time, which were also significantly lower compared to their control counterparts on the 3rd, 5th, and 7th days. Last, regarding the TEG results, a significant decrease in MA values during storage time was found in treated platelets (p<0.001), while these values were also lower than their control counterparts on the 3rd, 5th, and 7th days (p=0.010, p= 0.001, and p<0.001 respectively).

The main correlations between the results of the performed laboratory tests for the Mirasol-treated and control platelets are presented in **Tables III** and **IV**. In Mirasol-treated platelets, a significant association was detected between clot strength decrease, as reflected by the MCF and MA values, with glucose consumption, lactate production, annexin production, and pH decrease. The same correlations were either weaker or absent in control platelets.

## DISCUSSION

The results of this study indicate that PRT-treatment for platelets stored in PAS significantly affects their metabolic activity, as several changes in pH, glucose, LDH and blood gas levels were evident in PRT-treated platelets. Specifically, it was shown that the pH level of treated platelets progressively decreased over time, starting from the third day of the storage period. This is in accordance with the well-established observation that PRT treatment is associated with an increased metabolic activity of PCs, which could be attributed to the elevated glycolysis and the subsequent increased lactate production during the storage period. However, the pH levels in our study were higher than 6.4, which has been set as the threshold for product release by the European Council[20]. Moreover, it was found that glucose reserves in treated PAS platelets were exhausted on days 5 and 7, which is in line with the results of other studies[15,21,22-24]. This indicates that glucose levels in PAS-stored platelets could be a more valuable quality marker compared to pH, since pH levels remain stable over time due to PAS's inherent properties[25]. Last, LDH, a biomarker associated with platelet membrane integrity and platelet survival[26] did not increase until day 5 in both groups, but it was significantly increased from day 5 to day 7 in treated PCs, and to a lesser degree in control units. Similar to our findings, previous studies evaluating platelet changes also did not report any changes in LDH levels until day 5[15,22]. It must be noted though that the outcomes of this study are based on an *in vitro* assay during a 7-day storage period.

Regarding blood gas changes, pO2 levels remained stable, while pCO2 levels had a time-dependent decrease, mostly notable on the 7th day. A gradual decline in HCO3- values was also noted in both M and C samples, which was more evident in treated platelets. However, it is worth mentioning that there are contradicting findings in the literature regarding changes in blood gas levels, probably due to differences in assay temperatures and in the analyzers being utilized, as the riboflavin used in some of them interferes with results[24,27]. Generally, most metabolic markers demonstrated a significantly increased metabolism state in Mirasol treated PCs as compared to control ones.

Moreover, the results of this study indicate that PRT-treatment for platelets stored in PAS significantly affects their hemostatic capacity. Platelet aggregation, as has previously been reported, is being progressively reduced during the storage period[28,29]. In line with this, LTA with ADP agonist showed a significantly reduced aggregation over time both for treated and untreated platelet samples in our study. Ostrowski *et al*. observed an immediate reduction in ADP-induced aggregation in treated platelets, while most studies have reported

similar findings regarding the ADP induced aggregation between treated and control groups[30,31,32]. When PAS is used as a storage medium, there is a an additional loss of aggregation ability probably due to rapid desensitization of ADP receptors after release of granular ADP during storage, along with the lack of fibrinogen and vWF due to minimal plasma content[33-35]. However, in most studies evaluating the impact of PAS on platelet aggregation, older implementations were used such as PAS-II and it is not clear whether newer additive solutions would act differently. Last, Annexin-V showed a progressive and significant increase during storage for treated platelets, while this was not evident in untreated platelets.

Regarding the viscoelastic studies, the results of ROTEM analysis and TEG indicate that PRT treatment greatly affects the hemostatic capacity of treated platelets, probably due to PSL acceleration over storage time. Specifically, the CFT, MCF, A10 and MCE parameters of the EXTEM assay in ROTEM analysis showed a significant change in treated platelets during storage compared to control platelets, which did not demonstrate any notable change. The results of TEG analysis were in line with those of ROTEM analysis. Specifically, the maximum amplitude (MA) in rapid-TEG also demonstrated a significant reduction in Mirasol treated units both during storage time and compared with control PCs after day 3, while R and K parameters did not show any significant alteration. Rapid-TEG utilizes tissue factor to induce coagulation, making it comparable with EXTEM assay in ROTEM analyzer, as both activate the extrinsic pathway system which is the main in vivo coagulation contributor. Moreover, it is noteworthy that the hemostatic properties of control platelets suspended in plasma were significantly affected during storage period, as opposed to the control platelets in PAS in the current study. To the best of our knowledge, there are only a few studies evaluating the impact of PRT treatment on platelets through viscoelastic methods. Specifically, there are two studies using TEG, only one using ROTEM, while there is no study using both to assess PRT treated platelets' activity[30,36,37]. The results of these studies are in line with our findings. Ostrowski *et al*. found a reduction in MA values after the 8$^{th}$ storage day, while Ballester *et al*. also found significantly lower MA values between day 7 and 14 of the storage period. However, as opposed to these studies, clot strength alterations were evident in our study as early as day 3 in treated platelets. Moreover, Petrou *et al*. in the only study using ROTEM analysis, reported significantly altered A10, MCF and MCE values as in our study. However, Petrou *et al*[36] used plasma as a platelet suspension medium, thus we can assume that the observed changes in the ROTEM values are associated exclusively to the Mirasol treatment, and not to the type of medium, indicating a negative impact of Mirasol treatment on platelet hemostatic capacity. However, the strong association between clot strength decrease and metabolic parameters suggestive of increased PSL such as decreased pH levels and increased lactate production, and the absence of clot strength decline in control samples indicate a causal relationship between decreased platelet hemostatic activity and PRT treatment. In line with this, in a recent systematic review regarding the impact PRT-treatment on platelet activity, Tsalas *et al*. reported that despite the high heterogeneity in literature, there is a potential association between PRT and reduced aggregation response[38]. The critical issue to be clarified though is the time point at which this platelet dysfunction occurs and becomes clinically significant. The high heterogeneity regarding the time point that changes in clot strength start to be evident among the available studies may be related to the different assays, reagents, or suspension mediums that have been used in these studies. In a recent study, Petrou *et al*. reported a significant decrease over time in clot strength of control platelets suspended in plasma[36]. As opposed to the results of this study we found no change in clot strength over time in control platelets which were suspended in PAS. Therefore, PAS may be a more suitable medium in terms of platelet activity preservation. The prolongation of CFT in the treated platelets of our study, as opposed to that of Petrou *et al*, might also be due to the different suspension medium used. The activation of the coagulation cascade in this group due to PRT treatment leads to functional "exhaustion" of hemostatic capacity which becomes more intense because of the minimal plasma content of coagulation factors in PAS.

We acknowledge that there are certain limitations of our study. First, the number of recruited apheresis-platelet donors is relatively small. Moreover, an *in vitro* evaluation of the hemostatic and metabolic properties of the assessed platelets does not provide a completely accurate overview

of the hemostatic profile of platelets following their transfusion. However, this is one of the few, if not the only study assessing both metabolic and hemostatic profile of UV-treated platelets stored in T-PAS+.

## CONCLUSIONS

There is a lot of debate regarding the impact of PRT on the hemostatic capacity of treated platelets, the ideal suspension medium, and the recommended storage time for an optimal therapeutic effect of transfused platelets. Our findings indicate that Mirasol treatment and storage alter the hemostatic capacity of treated platelets in a many-sided way, prompting questions about the proper handling of these platelet components. Based on our findings, the metabolism of PRT treated platelet is accelerated, while their hemostatic capacity is negatively affected by PRT. Moreover, clot strength was found to be adversely affected by the longer storage time in treated platelets, while the hemostatic capacity of untreated platelets stored in PAS was maintained unaffected. However, it is yet to be investigated whether the negative impact of PRT on the hemostatic capacity of platelets affects their clinical efficacy. The clinical relevance of our findings should be investigated in large clinical studies, while further research is warranted regarding the optimal suspension medium and the ideal storage period of PRT-treated platelets. Last, development of newer biomarkers for evaluation of their *in vitro* viability and activity would be valuable in order to determine the optimal time for transfusion.



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## AUTHORSHIP CONTRIBUTIONS

ST, and AET conceived the study. All Authors contributed to the design of the study protocol. ST, EP, SM, EL, AV, AK, SF, KAT, RS, and SK conducted the work and collected the data. AGT, and DP performed the statistical analysis. All the Authors contributed to the interpretation of data for the work. ST, AGT, EP, and AET drafted the manuscript. All Authors critically revised the paper for important intellectual content and approved the final version to be published.

*The Authors declare no conflicts of interest.*

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