

Good hemostatic effect of platelets stored at 4°C in an in vitro model of massive blood loss and thrombocytopenia

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Abstract

This study compared the corrective effects of storage of platelets at 4°C and at 22°C in an in vitro model of massive blood loss and thrombocytopenia to provide an experimental basis for the storage of platelets for clinical applications.

In vitro model of massive blood loss and thrombocytopenia were constructed by the in vitro hemodilution method and cell washing method. Using storage of platelets at 4°C (1, 3, 5, 7, 10, 14 days) and at 22°C (1, 3, 5 days) to correct the coagulation condition of the different models, by thromboelastography and by routine blood indices.

① Platelets stored at 4°C (1, 3, 5, 7, 10, 14 days) and at 22°C (1, 3, 5 days) to correct the in vitro model of massive blood loss. Platelet count results improved from 17 to 27 × 10⁹/L to greater than 120 × 10⁹/L for 4°C storage, and 20 to 27 × 10⁹/L to greater than 120 × 10⁹/L for 22°C storage. Thromboelastography maximum amplitude (TEG-MA) results improved from 8.8 to 15.4 mm to greater than 43 mm for 4°C storage, and 12.2 to 14.4 mm to greater than 44.8 mm for 22°C storage. Thromboelastography reaction time values decreased from 9.9–24.9 minutes to 3.8–5.5 minutes for 4°C storage, and 9.9–22.7 minutes to 4.3–4.5 minutes for 22°C storage. ② Platelets stored at 4°C (1, 3, 5, 7, 10, 14 days) and at 22°C (1, 3, 5 days) to correct the in vitro model of thrombocytopenia. Platelet count results improved from 12 to 34 × 10⁹/L to greater than 99 × 10⁹/L for 4°C storage, and 12 to 34 × 10⁹/L to greater than 120 × 10⁹/L for 22°C storage. TEG-MA results improved from 21.4 to 32.1 mm to greater than 49.1 mm for 4°C storage, and 21.4 to 31.6 mm to greater than 50.5 mm for 22°C storage.

Platelets stored at 4°C and 22°C have the same correcting effect for 1, 3, and 5 days. Platelets stored at 4°C for 7 to 14 days have similarly hemostatic effect on the in vitro model of massive blood loss and thrombocytopenia.

Abbreviations: APTT = activated partial thromboplastin time, FFP = fresh frozen plasma, FIB = fibrinogen, Hb = hemoglobin, MCV = mean corpuscular volume, MPV = mean PLT volume, PCT = plateletcrit, PDW = PLT distribution width, PLT = platelet, PT = prothrombin time, TEG = thromboelastography, TEG-α = thromboelastography alpha, TEG-CI = thromboelastography coagulation indice, TEG-K = thromboelastography kinetics, TEG-MA = thromboelastography maximum amplitude, TEG-R = thromboelastography reaction time, WBC = white blood cell.

Keywords: cold storage, in vitro, model of massive blood loss, platelet, thrombocytopenia

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J-CY and F-HL contributed equally to this paper.

The authors declare that they have no conflicts of interest relevant to the manuscript submitted.

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1. Introduction

Platelet (PLT) transfusion plays an irreplaceable role in the prevention and treatment of thrombocytopenia, as therapy for surgical trauma, and during rescue treatment of patients with massive blood loss. In China, PLTs are usually stored at a temperature of 20 to 24°C, with a storage time limit of 5 days.^[1] This is because, PLTs stored at 22°C are prone to bacterial contamination and loss of PLT function, thereby limiting PLT storage time at this temperature to 5 to 7 days.^[2–14] This is the basis for this study, on how to resolve the clinical shortage of PLTs, and how to establish a convenient PLT storage method that also ensures long-term storage. This will address the root cause of the clinical PLT shortage problem.

Recently, some studies found that PLTs stored at 4°C have many advantages such as longer time of preservation, low levels of bacterial contamination,^[15] strong aggregation effect,^[16] and good hemostatic function.^[17,18,19] Some investigations reported that PLTs in whole blood stored at low temperatures could survive for 10 days in the circulation,^[20,21] and could be preserved for 5 to 7 days, and 14 days in vitro.^[19,22,23] The results of a series of experiments that we conducted on PLTs stored at 4°C in vitro were

as follows^[24]: PLTs stored at 4°C for 10 to 14 days showed better PLT count, cellular morphology, and PLT membrane structure and cytoplasm than PLTs stored at 22°C for 5 days; PLTs stored at 4°C had slower metabolism, including less production of lactic acid, slower decrease of pH, lower consumption of glucose, and a higher recovery rate of the PLT hypotonic shock reaction rate compared with PLTs stored at 22°C within the same storage period; PLTs stored at 4°C had higher degree of aggregation, a stronger hemostatic function, and a higher activation state than the latter, and could be preserved for 10 to 14 days.

To verify the results of the above-mentioned experiments, under conditions unsuitable for *in vivo* experiments, in this study, we constructed an *in vitro* model of thrombocytopenia and massive blood loss where PLTs stored at 4°C were used for correction. This was compared with correction by PLTs stored at 22 to 24°C, to analyze and verify the appropriate effect of cold storage of PLTs, and to provide an experimental basis for early clinical applications.

2. Methods

2.1. Study design

Based on the results of previous studies,^[22–24] the correction effect of PLT preservation in cold storage in the *in vitro* model of thrombocytopenia^[25] has been verified. Thromboelastography (TEG) indices and routine blood indices were used to evaluate blood coagulation function, anemia corrective effect, and PLT counts on an *in vitro* model of massive blood loss and thrombocytopenia (see experimental flow chart; Fig. 1).

2.2. Volunteer inclusion criteria

Without inducing compensation or any conflict of interest, participating volunteers complied with the clear medical and legal agreements by providing informed consent. The inclusion criteria for the volunteers were as follows: absence of susceptibility to relevant disease or of intake of medications, which could affect PLT function/count within the preceding 3 years or in the preceding 15 days, respectively. The volunteers were aged 25 to 51 years and comprised 10 females and 10 males.

2.3. Source of blood supplements

In this study, suspended red blood cells (RBCs) and fresh frozen plasma (FFP) were provided by the Blood Center of Shaanxi Province. All manually-prepared PLTs were provided by the Department of Transfusion Medicine, Xijing Hospital. The blood samples to be used for scientific research purposes were approved by the Blood Center of Xijing Hospital, Shaanxi Province, and the ethics committee of Shaanxi Provincial People’s Hospital. Written informed consent was obtained from all donors before enrollment in this study. All blood donors met the requirement as regulated in the health standard for blood donors—the standard developed by the Ministry of Health, China.

2.4. Manual preparation of PLTs

PLTs were manually prepared by the buffy coat method. Each bag of PLTs (volume, 200 mL) originated from 8 to 10 donors and was divided into 2 sub-bags that were separately stored at 4°C and 22°C, respectively. The PLTs consisted of PLT concentrates in plasma, with no PLT additive solution, consisting of white blood cell (WBC)-filtered PLT concentrate.^[24]

2.5. Experimental devices and test indicators

Routine blood indices were assessed using the SysmexXT-1800i fully automatic blood analyzer. Routine blood parameters included WBC count, RBC count, hemoglobin (Hb) content, mean corpuscular volume (MCV), PLT count, plateletcrit (PCT), mean PLT volume (MPV), and PLT distribution width (PDW). Blood coagulation function was analyzed using a Thrombelastograph (Hemoscope Corp, Braintree, MA). TEG parameters were reaction times (R or R-time), kinetic times (K or K-time), angle (α), maximum amplitude (MA), and coagulation index (CI).

2.6. Study method

2.6.1. Correction effects of manual PLTs on the *in vitro* model of massive blood loss

2.6.1.1. Construction of the model for *in vitro* massive blood loss. A model of *in vitro* massive blood loss was established by the *in vitro* hemodilution method employed in our previous

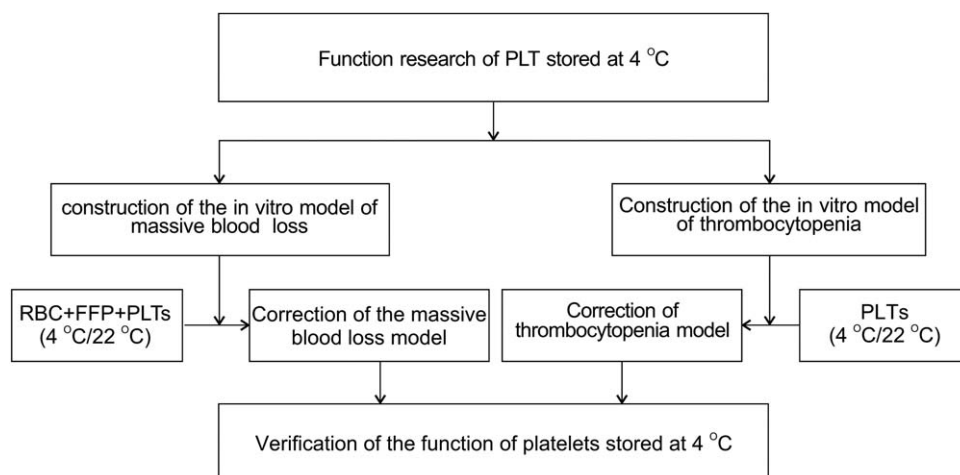


Figure 1. Study design experimental flow chart.

study.^[2,5] Preparation of the in vitro saline hemodilution model and measurement of routine blood indices and coagulation indices were as follows: the blood sample was diluted with normal saline in ratios of 1:9.

2.6.1.2. Dividing and preprocessing of blood components used for correcting in vitro massive blood loss. In this experiment, massive blood loss was corrected with the blood components, which consisted of RBCs, FFP, and manually prepared PLTs. The RBCs originated from volunteers with similar blood types. In this experiment, 5 bags of RBCs were taken out from storage at 4°C and slightly shaken horizontally for 2 minutes. Furthermore, a 6 mL blood sample in each of the RBCs bags was collected under sterile conditions for later experiments.

FFP also originated from volunteers with similar blood types. A 3-mL blood sample in each of the frozen plasma bags was collected for a later experiment.

A total of 10 samples of manually-prepared PLTs. The PLTs stored at 4°C were labeled as PLT-4-1, PLT-4-2, PLT-4-3, PLT-4-4, and PLT-4-5. The PLTs stored at 22°C were labeled as PLT-22-1, PLT-22-2, PLT-22-3, PLT-22-4, and PLT-22-5. PLT-4-1 and PLT-22-1 were obtained from the same subject. Similarly, PLT-4-2 and PLT-22-2, PLT-4-3 and PLT-22-3, PLT-4-4 and PLT-22-4, and PLT-4-5 and PLT-22-5 were obtained from the same source. The resulting 10 bags of PLT samples (5 bags stored at 4°C and 5 bags stored at 22°C) were placed at room temperature for 5 minutes, and were then slightly and horizontally shaken to enable a homogeneous mixing, on the day of the experiment. Ten clean and anticoagulant-free tubes were labeled with the same numbers as the PLTs. After the soft pipes of the PLT storage bags were sterilized, a 2 mL portion of the PLTs was

drawn into each of the labeled tubes. With the soft pipes being immediately subjected to thermal sealing and then cut, the PLT storage bags were placed back in their individual, previous storage conditions.

2.6.1.3. Procedures for correcting in vitro massive blood loss. The experiment was conducted in 2 groups: an experimental and a control group. In the experimental group, RBCs, plasma, and PLTs stored at 4°C were added at certain ratios (RBCs: FFP: PLTs=1:1:1) to the blood samples used for the blood loss models. In the control group, RBCs, plasma, and PLTs stored at 22°C were similarly added in the same ratios (RBCs: FFP: PLTs= 1:1:1) to the blood samples used for the blood loss models. The ratios and the amount of the added RBCs, plasma, and PLTs were selected based on our previous study.^[2,5,26] The detailed experimental procedures are shown in Figure 2.

2.6.1.4. Laboratory measurements before and after correction. In the experimental group,

- (1) routine blood examination indices of PLTs at different storage periods,
- (2) routine blood examination indices and TEG indices of the original blood of volunteers,
- (3) the in vitro models of blood loss (before and after PLT correction) were measured on day 1, 3, 5, 7, 10, and 14.

In the control group,

- (1) routine blood examination indices and TEG indices of the original blood of volunteers,
- (2) the in vitro models of blood loss (before and after PLT correction) were measured on day 1, 3, and 5.

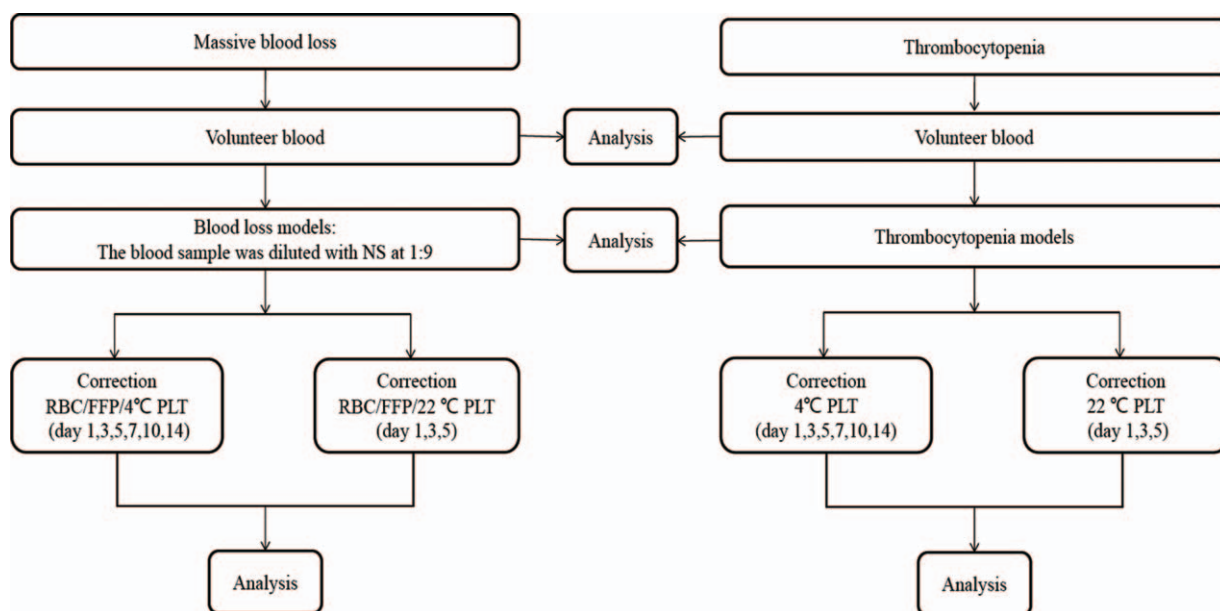


Figure 2. A flowchart showing the correction of models of in vitro blood loss and thrombocytopenia. Analysis of routine blood parameters: WBC, RBC, Hb, MCV, PLT, PCT, MPV, PDW. Analysis of TEG parameters: TEG-R, TEG-K, TEG-alpha, TEG-MA, TEG-CI. Hb=haemoglobin, MCV=mean corpuscular volume, MPV=mean PLT volume, PCT=plateletcrit, PDW=PLT distribution width, PLT=platelet, RBC=red blood cell, TEG=thromboelastography, TEG-α=thromboelastography alpha, TEG-CI=thromboelastography coagulation indice, TEG-K=thromboelastography kinetics, TEG-MA=thromboelastography maximum amplitude, TEG-R=thromboelastography reaction time, WBC=white blood cell.

2.6.2. Correction effects of PLTs on the *in vitro* model of thrombocytopenia

2.6.2.1. Construction of the *in vitro* model for thrombocytopenia—cell washing method. On the day of the experiment, 14 mL of fasting venous blood samples were collected from the volunteers and stored in 7 Vacutainer tubes containing sodium citrate as an anticoagulant. The first Vacutainer tube had 2 mL of venous blood discarded (to avoid interference with skin tissue factors) while the remaining 12 mL of blood was distributed into the remaining 6 Vacutainer tubes, with whole blood to sodium citrate anticoagulant ratio of 9:1. Six tubes of whole blood from the same volunteers were symmetrically centrifuged for 4000 rpm for 5 minutes. Afterward, the upper plasma layer in each of the 6 tubes was removed and transferred into a dry Vacutainer tube. Care was taken to avoid disturbing the PLT level during the process. Two hematocrit volumes of physiological saline were added to the remaining hematocrit and mixed gently by pipetting before centrifugation at 1000g for 3 minutes. The supernatant was discarded and the washing process was repeated to sufficiently remove the PLTs. Washed erythrocytes were collected into 1 tube together with the pre-kept plasma layer at a volume ratio of hematocrit to plasma of 1:1. Mild pipetting was used to mix the contents.

2.6.2.2. Correction method for the *in vitro* model of thrombocytopenia. There were 2 groups in the experiment. The experimental group and control groups were the correction groups using PLTs at 4°C and at 22°C, respectively. Six-milliliter blood samples from the constructed *in vitro* model of thrombocytopenia were distributed equally into 4 tubes (1.5 mL/tube) and each of these tubes had 225 μ L of PLTs at 4°C added accordingly (8:1 correction ratio). PLTs at 22°C were added to the remaining 2 tubes.

2.6.2.3. Laboratory measurements before and after correction. In the experimental group,

- (1) routine blood examination indices of PLTs at different storage periods,
- (2) routine blood examination indices and TEG indices of the original blood of volunteers,
- (3) the *in vitro* models of thrombocytopenia (before and after PLT correction) were measured on day 1, 3, 5, 7, 10, and 14.

In the control group,

- (1) routine blood examination indices and TEG indices of the original blood of volunteers,
- (2) the *in vitro* models of thrombocytopenia (before and after PLT correction) were measured on day 1, 3, and 5.

2.7. Statistical analysis

The statistical analyses were conducted using SPSS (IBM Corp, Armonk, NY). Variable data were expressed as the mean \pm standard deviation ($\bar{x} \pm s$), and the means of 2 groups were compared using the Student *t* test, while the means of multiple variables were compared using 1-way analysis of variance (ANOVA). Replicate measurement data were analyzed using repeated measures ANOVA.

3. Results

3.1. Construction and correction of models of massive blood loss

3.1.1. Construction of models of massive blood loss. As shown in Table 1, there were statistically significant differences in routine blood indices including WBC, RBC, Hb, PLT, and PCT monitored at various time points (days 1, 3, 5, 7, 10, and 14) between the original blood of volunteers and the blood loss models ($P < .05$). The results showed that WBC, RBC, Hb, PLT, and PCT decreased in the blood loss models compared with the original blood of volunteers.

There were statistically significant differences in TEG parameters (*R*, α , and MA) at the various monitoring time points (days 1, 3, 5, 7, 10, and 14) between the original blood of volunteers and the blood loss models ($P < .05$), with the *R* increasing, and α and MA decreasing in the blood loss models. *K* and CI were not detectable, showing that the hemagglutination activity was low.

3.1.1.1. The correction effects of the models of *in vitro* massive blood loss. Hb, PLT, *R*, and MA were selected as the main indicators for the assessment of the correction efficiency of the massive blood loss models as shown in Table 2 (Fig. 3A–C). There was no statistically significant difference in the corrected Hb, PLT, *R*, and MA values of the blood loss models established with the same blood samples between the 2 groups for the storage days 1, 3, and 5. The results showed the 2 groups have the same practical effect *in vitro* model of massive blood loss. Similarly, there was no difference ($P > .05$) in other corrected parameters (WBC, RBC, MCV, PCT, WPV, PDW, *K*, α , and CI) between the 2 groups for the storage days 1, 3, and 5.

As shown in Table 2 (Fig. 3A–C), the PLT results showed statistically significant difference ($P < .05$) after the correction of the blood loss models with PLT-4, within 14 days of PLTs at 4°C ($P4(14) = 0.001$), therefore, all the corrected values of PLT showed correction efficiency. Furthermore, there was no statistically significant difference ($P > .05$) in the corrected value at day 1, 3, 5 between PLT-4 and PLT-22. There was a statistically significant difference ($P < .05$) in the value of PLT count corrected with PLT-22, within 5 days of PLTs at 22°C ($P1(5) = 0.014$).

After the correction of the blood loss models with PLT-4, there was no statistically significant difference ($P > .05$) in MA within 14 days of PLTs at 4°C ($P4(14) = 0.824$). There was no statistically significant difference ($P > .05$) in the corrected MA values within 5 days of PLTs at 22°C ($P1(5) = 0.324$). There were statistically significant differences in the values of *R* and CI at days 7 to 14 at 4°C. However, the corrected values of *R* and CI fell within the normal ranges. There was no statistically significant difference ($P > .05$) between the value of *R* and CI after the corrected value at day 5 obtained with PLT-22 (Table 2). There was no statistically significant difference ($P > .05$) in the value of MA between PLT-22 stored at 5 days and PLT-4 at 7, 10, 14 days. There were statistically significant differences ($P < .05$) in the other corrected parameters (WBC, RBC, Hb, MCV, PCT, WPV, PDW, *K*, and α) within 10 and 14 days of PLTs at 4°C, and all the parameters showed correction efficiency.

3.2. Construction and correction of the *in vitro* model of thrombocytopenia

3.2.1. Construction of the *in vitro* model of thrombocytopenia. As shown in Table 3 (Fig. 4A–D), there were statistically significant differences in routine blood indices and TEG including

Table 1
The establishment of models of massive blood loss and the changes in the model indicators (n=20, $\bar{x} \pm SD$).

Test item	Blood loss models	1 d	3 d	5 d	7 d	10 d	14 d
WBC, $\times 10^9/L$	Original	6.14 \pm 0.89*	6.02 \pm 2.45*	6.67 \pm 1.26*	6.67 \pm 1.33*	7.36 \pm 0.39*	7.29 \pm 0.23*
	Model	0.56 \pm 0.06	0.51 \pm 0.17	0.72 \pm 0.20	0.62 \pm 0.14	0.52 \pm 0.01	0.54 \pm 0.01
RBC, $\times 10^{12}/L$	Original	5.04 \pm 0.29*	3.97 \pm 0.83*	4.3 \pm 0.17*	4.57 \pm 0.26*	4.6 \pm 0.25*	4.31 \pm 0.26*
	Model	0.48 \pm 0.01	0.39 \pm 0.06	0.28 \pm 0.20	0.43 \pm 0.02	0.36 \pm 0.01	0.36 \pm 0.01
Hb, g/L	Original	154 \pm 13*	113 \pm 34*	121 \pm 18*	146 \pm 7*	138 \pm 5*	124 \pm 23*
	Model	15 \pm 1	11 \pm 3	12 \pm 2	14 \pm 1	11 \pm 1	11 \pm 2
MCV, fL	Original	84.7 \pm 1.5	80.8 \pm 4.2	81 \pm 4.9	87.1 \pm 7.2	84.3 \pm 0.1	81.4 \pm 5.6
	Model	88.5 \pm 1.8	84.2 \pm 5.9	84.3 \pm 5.4	91.9 \pm 4.6	85.9 \pm 0.3	84.5 \pm 6.3
PLT, $\times 10^9/L$	Original	165 \pm 25*	168 \pm 18*	204 \pm 35*	143 \pm 60*	156 \pm 6*	264 \pm 61*
	Model	20 \pm 0	24 \pm 8	27 \pm 9	18 \pm 2	17 \pm 1	27 \pm 4
PCT, %	Original	0.17 \pm 0.01*	0.17 \pm 0.008*	0.23 \pm 0.01*	0.14 \pm 0.05*	0.17 \pm 0.01*	0.29 \pm 0.07*
	Model	0.02 \pm 0	0.03 \pm 0.01	0.03 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.03 \pm 0.01
MPV, fL	Original	10.5 \pm 2.4	11 \pm 0.8	11.6 \pm 0.8	10.6 \pm 0.3	11.1 \pm 1.1	11 \pm 0.1
	Model	9.6 \pm 1.6	9 \pm 0.1	9.1 \pm 0.4	8.6 \pm 0.1	9 \pm 1.1	9.5 \pm 0.2
PDW, %	Original	13.6 \pm 5.4	13.8 \pm 1.9	16.1 \pm 3.3	13.1 \pm 0.2	15.2 \pm 4.3	13.2 \pm 0.6
	Model	9.6 \pm 3.4	10.2 \pm 1.9	8.5 \pm 0.6	9.4 \pm 0.1	10.4 \pm 3.4	10.6 \pm 0.9
R, min	Original	7.2 \pm 0*	6.8 \pm 0.3*	4.7 \pm 3.3	5.3 \pm 1.5*	5.3 \pm 1.5*	4.6 \pm 0.8*
	Model	22.7 \pm 2.5	21.3 \pm 0.5	9.9 \pm 3.5	24.9 \pm 6.3	21.8 \pm 4.9	13.6 \pm 1.3
K, min	Original	1.9 \pm 0.3	1.5 \pm 0.1	1.4 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.1	1.3 \pm 0.1
	Model	ND	ND	ND	ND	ND	ND
α , dag	Original	69.3 \pm 2.7*	73.7 \pm 0.5*	74 \pm 0*	71 \pm 2.1*	71 \pm 2.1*	75.2 \pm 1.1*
	Model	42.1 \pm 3.9	42.8 \pm 0.6	42.1 \pm 0.4	39.1 \pm 0.4	39.9 \pm 1.2	43.1 \pm 0.8
MA, mm	Original	59.7 \pm 3.6*	67 \pm 4*	69.6 \pm 0.3*	59.3 \pm 0.6*	59.3 \pm 0.6*	65.1 \pm 10.7*
	Model	12.7 \pm 5.5	14.4 \pm 0.1	12.2 \pm 1.4	8.8 \pm 1.3	9.9 \pm 1.6	15.4 \pm 1.6
CI	Original	-0.7 \pm 0.2	1.1 \pm 0.4	2.8 \pm 2.1	0.8 \pm 1.1	0.8 \pm 1.1	2.4 \pm 2
	Model	ND	ND	ND	ND	ND	ND

α = alpha, CI = coagulation indices, Hb = hemoglobin, K = kinetics, MA = maximum amplitude, MCV = mean corpuscular volume, MPV = mean platelet volume, ND = data not detectable, PCT = plateletcrit, PDW = platelet distribution width, PLT = platelets, R = reaction time, RBC = red blood cell, WBC = white blood cell.

*The comparison between the original blood of volunteers and the blood loss models ($P < .05$).

PLT, WBC, R, K, MA, and CI at various time points (days 1, 3, 5, 7, 10, and 14) between the original blood of volunteers and the in vitro model of thrombocytopenia ($P < .05$), with the model showing reduced PLT, WBC, MA, R, and CI and increase in K values.

3.2.2. Correction effects on the in vitro model of thrombocytopenia. Parameters of routine blood and TEG were used to evaluate blood coagulation function on the in vitro thrombocytopenia model. The PLT counts results of the correction effects of PLTs stored at 4°C and at 22°C, showed that on days 1, 3, 5, the PLT values of the in vitro thrombocytopenia model were $24 \pm 8 \times 10^9/L$, $12 \pm 5 \times 10^9/L$, and $34 \pm 14 \times 10^9/L$, respectively (Table 4). After the PLTs from the same blood sample were added, the PLT values were $149 \pm 42 \times 10^9/L$, $130 \pm 45 \times 10^9/L$, and $150 \pm 34 \times 10^9/L$ for 4°C storage; and $123 \pm 35 \times 10^9/L$, $127 \pm 30 \times 10^9/L$, and $142 \pm 33 \times 10^9/L$ for 22°C storage, on days 1, 3, 5, respectively, with no statistically significant difference between groups on the same day ($P > .05$). Under the same condition, the thromboelastography maximum amplitude (TEG-MA) value showed no statistically significant difference ($p > 0.05$). Other indices such as WBC, RBC, Hb, MCV, PCT, MPV, R, K, α , and CI values similarly showed no difference in correction effects between groups ($P > .05$). There were statistically significant differences in the PDW values between groups on days 1 and 5 ($P < .05$).

As shown in Table 4, when PLT-4 was used for correction of the in vitro thrombocytopenia model, there was statistically significant difference in PLT values within 14 days at 4°C (P

(14) = 0.027), there was no statistically significant difference in correction effects within 10 days ($P(10) = 0.515$). There was no statistically significant difference when compared with the correction results of PLT-22 within 5 days ($P(5) = 0.490$). There was no statistically significant difference ($P > .05$) in PLT values between PLT-22 stored at 5 days and PLT-4 at 7, 10, 14 days, respectively.

There was no statistically significant difference in MA values within 7 days at 4°C ($P(7) = 0.123$), whereas MA values within 10 and 14 days remained 49.1 ± 6 and 50.6 ± 2.8 . There was no statistically significant difference within 5 days ($P(5) = 0.310$) when PLT-22 was used for correction. No statistically significant differences in the other indices (RBC, Hb, MCV, PCT, MPV, PDW, R, K, α , and CI) were observed within 10 to 14 at 4°C ($P > .05$).

4. Discussion

The main reason why massive blood loss leads to coagulation disorders is the decrease in PLT and blood clotting factors caused by dilution and consumption. The adequate and early supply of PLT and blood clotting factors play a pivotal role in improving the survival of patients with massive blood transfusion.^[26]

The current standard method for the storage of PLTs is to shake and store at 20 to 24°C for 5 days. Due to the short shelf life of PLTs, its storage is limited, and shortages in PLT supply makes it difficult to meet the volume demand for treatment in clinical practice. Cold storage is receiving renewed interest due to its potential to extend shelf life,^[27] while increasing the hemostatic

Table 2
The comparison of the correction of massive blood loss models with RBCs+FFP+PLT-4/PLT-22 (n=20, $\bar{x} \pm SD$).

Test item	Group	1 d	3 d	5 d	7 d	10 d	14 d	p1 (5)	p2 (7)	p3 (10)	p4 (14)
WBC, $\times 10^9/L$	PLT-4	2.9 \pm 0.25	3.1 \pm 0.27	3.21 \pm 0.17	3.03 \pm 0.13 [†]	2.82 \pm 0.31 [†]	2.84 \pm 0.1 [†]	0.095	0.142	0.084	0.001
	PLT-22	2.9 \pm 0.31	3.12 \pm 0.55	3.21 \pm 0.26				0.47			
RBC, $\times 10^{12}/L$	PLT-4	1.66 \pm 0.25	1.51 \pm 0.41	2.13 \pm 0.18	1.79 \pm 0.16 [†]	1.81 \pm 0.38 [†]	1.79 \pm 0.24 [†]	0.001	0.001	0.001	0.001
	PLT-22	1.64 \pm 0.31	1.32 \pm 0.13	2.23 \pm 0.16				0.001			
Hb, g/L	PLT-4	51 \pm 9	47 \pm 11	61 \pm 3	50 \pm 8 [†]	59 \pm 13 [†]	56 \pm 7 [†]	0.001	0.002	0.004	0.001
	PLT-22	56 \pm 19	41 \pm 4	62 \pm 2				0.036			
MCV, fL	PLT-4	90 \pm 0.5	94.1 \pm 0.4	87.5 \pm 2.5	88.2 \pm 14	96.1 \pm 0.9 [†]	90.5 \pm 3.7 [†]	0.001	0.043	0.008	0.001
	PLT-22	90 \pm 0.2	94.3 \pm 0.7	87.6 \pm 2.3				0.001			
PLTs, $\times 10^9/L$	PLT-4	128 \pm 18	128 \pm 17	160 \pm 8	162 \pm 9	148 \pm 18 [†]	141 \pm 31 [†]	0.001	0.001	0.001	0.001
	PLT-22	127 \pm 18	126 \pm 19	161 \pm 16				0.014			
PCT, %	PLT-4	0.13 \pm 0.02	0.14 \pm 0.02	0.15 \pm 0.01	0.16 \pm 0.01	0.15 \pm 0.02	0.12 \pm 0.01 [†]	0.006	0.002	0.003	0.001
	PLT-22	0.12 \pm 0.02	0.14 \pm 0.02	0.16 \pm 0.02				0.075			
MPV, fL	PLT-4	9.9 \pm 0.3	10.9 \pm 0.2	9.6 \pm 0.5	10.3 \pm 0.1	10.3 \pm 0.1	9.5 \pm 1	0.001	0.001	0.001	0.003
	PLT-22	9.8 \pm 0.4	10.8 \pm 0.4	9.9 \pm 0.6				0.01			
PDW, %	PLT-4	10.8 \pm 0.8	12.9 \pm 0.9	10.6 \pm 1	12.1 \pm 0.3	12 \pm 0.3	10.6 \pm 2	0.001	0.001	0.001	0.001
	PLT-22	10.8 \pm 0.7	13.9 \pm 1.2	11.5 \pm 1.5				0.003			
R, min	PLT-4	4.4 \pm 0.3	3.8 \pm 1.7	4.3 \pm 0.8	5.5 \pm 0.4	4.6 \pm 0.4	5.2 \pm 0.4	0.869	0.03	0.039	0.002
	PLT-22	4.5 \pm 0.2	4.7 \pm 0.2	4.3 \pm 0.6				0.175			
K, min	PLT-4	2.2 \pm 0.5	2.1 \pm 0.2	2.3 \pm 0.5	2.5 \pm 0.2 [†]	2.7 \pm 0.1 [†]	2.6 \pm 0.1 [†]	0.748	0.284	0.03	0.005
	PLT-22	2.2 \pm 0.4	2.3 \pm 0.4	2 \pm 0.1				0.266			
α , dag	PLT-4	68.6 \pm 3.8	69.7 \pm 0.9	68.2 \pm 3.2	67.7 \pm 1.7 [†]	65.1 \pm 1.4 [†]	65.6 \pm 1.6 [†]	0.49	0.376	0.021	0.006
	PLT-22	68.9 \pm 2	68.7 \pm 2.7	72.4 \pm 3.2				0.086			
MA, mm	PLT-4	45 \pm 7.4	51.1 \pm 2.7*	51.5 \pm 2.4	46 \pm 3.2	44 \pm 3.6	43.3 \pm 4.7	0.143	0.162	0.093	0.824
	PLT-22	47.2 \pm 9	44.8 \pm 2.5	50.4 \pm 2.9				0.324			
CI	PLT-4	-1 \pm 0.4	0.3 \pm 0.1*	0.5 \pm 0.3	-1.6 \pm 0.7 [†]	-1.5 \pm 0.7 [†]	-1.9 \pm 0.6 [†]	0.05	0.004	0.001	0.003
	PLT-22	-0.6 \pm 1.3	-1.1 \pm 0.5	0.3 \pm 0.5				0.074			

PLT-4: These were the data of PLT stored at 4°C after the correction of the models of massive blood loss, while PLT-22 represents the data of the PLT stored under shaking at 22°C after the correction of the models of massive blood loss.

α = alpha, CI = coagulation indice, FFP = fresh frozen plasma, Hb = hemoglobin, K = kinetics, MA = maximum amplitude, MCV = mean corpuscular volume, MPV = mean platelet volume, PCT = plateletcrit, PDW = platelet distribution width, PLT = platelets, R = reaction time, RBC = red blood cell, RBCs = red blood cell suspension, WBC = white blood cell.

* There was a statistically significant difference ($P < .05$) between the 2 groups after the correction at the same storage day.

[†] PLT-22 stored at 5 d was compared with PLT-4 at 7, 10, 14 d, respectively ($P < .05$). P1 (5) at 4°C was testified by repeated measurement from storage 1 to 5 d, and P1 (5) at 22°C was also testified by repeated measurement from storage 1 to 5 d. P2 (7) at 4°C was testified by repeated measurement from storage 1 to 7 d. P3 (10) at 4°C was testified by repeated measurement from storage 1 to 10 d. P4 (14) at 4°C was testified by repeated measurement from storage 1 to 14 d.

effectiveness of PLTs.^[28–30] Additionally, there is a reduction in bacterial growth rates,^[31] both in vitro and in vivo.^[28,29,32–33] These features would be beneficial in reducing the logistical burden of supplying rural areas and military medical centers. Thus, the problem of PLT shortage during transfusion could be resolved. In this study, we constructed an in vitro model of

thrombocytopenia and massive blood loss where PLTs stored at 4°C were used for correction.

To ensure that the Hb levels, PLT counts, and blood clotting function after the dilution would be maintained within normal ranges^[25,26] (for instance, Hb 60–80 g/L, PLT count of 50–75 $\times 10^9/L$, and TEG-MA >40 mm), we employed Hb, PLT count, R,

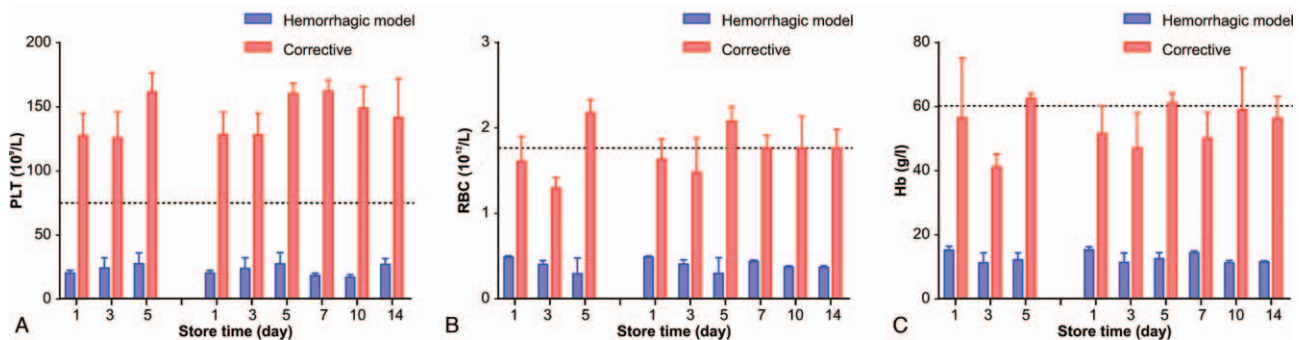


Figure 3. The routine blood before and after the correction of the models of in vitro massive blood loss. Dashed lines (— — — —): Appropriate standard of in vitro blood loss models. With reference to the current international guidelines for massive transfusion and surgical blood transfusion, we defined the following critical criteria for the administration of the blood supplements after in vitro hemodilution: Hb of 60 to 80 g/L, for RBC suspension; FIB of 0.8 to 1.0 g/L or PT or APTT 1.5 times the normal, for FFP; and PLT count of 50 to 75 $\times 10^9/L$ or TEG-MA of <40, for apheresis PLT. APTT = activated partial thromboplastin time, FFP = fresh frozen plasma, FIB = fibrinogen, Hb = haemoglobin, PLT = platelet, PT = prothrombin time, RBC = red blood cell, TEG-MA = thromboelastography maximum amplitude.

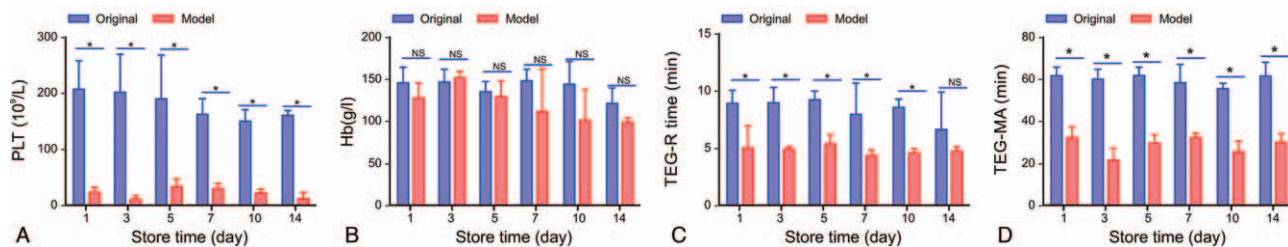


Figure 4. Blood routine indices changes in the in vitro model of thrombocytopenia.

and MA as the main indicators to assess the correction efficiency of blood loss models. We corrected the models of massive blood loss with RBCs, FFP, and PLT stored under different conditions, and found that the corrected PLT counts within 14 days of storage at 4°C showed correction efficiency. These values did not differ significantly ($P > .05$) from the values at day 5 of the storage at 22°C and also showed correction efficiency.

The corrected MA values of PLTs within 14 days of storage at 4°C and the values within 5 days of storage at 22°C did not differ significantly ($P > .05$). Although there were statistically significant differences in the values of R and CI within 7 to 14 days of storage at 4°C, the corrected values of the 2 parameters all fell within the normal ranges, indicating good correction efficiency. The K and CI values of the same model were not detectable, but all fell within normal ranges after correction. Similarly, the other parameters (WBC, RBC, Hb, MCV, PCT, WPV, PDW, and α) all showed correction efficiency.

We compared the use of PLTs stored at 4°C and 22°C on the correction of the thrombocytopenia model, and the results were consistent with the in vitro experiments conducted using PLTs stored at 4°C. In the latter experiments, PLTs stored for 10 TO 14 days at 4°C showed better hemostatic ability than PLTs stored at 22°C.

The main aim of PLT transfusion is for preventive purposes and to achieve hemostasis. Prevention requires PLT transfusion into the body to maintain a certain level for effective PLT functions. This is used in thrombocytopenia caused by decrease in blood formation in the bone marrow as can be seen in leukemia, or during radiotherapy and/or chemotherapy. Hemostasis requires PLT transfusion into the body for the immediate arrest of bleeding. This situation is mostly seen in patients with good blood-forming ability in the bone marrow, as occurs in conditions such as trauma, and hemorrhage in which bleeding occurs due to a decrease in peripheral PLT numbers. In such situations, transfused PLTs are required for high hemostatic

Table 3
Construction of the in vitro model of thrombocytopenia (n=20, $\bar{x} \pm SD$).

Test item	Original/ model	1 d	3 d	5 d	7 d	10 d	14 d
WBC, $\times 10^9/L$	Original	5.8±1.5*	6±1.5*	6.6±1.8*	6.4±2.2*	5.3±1.4*	5.3±1.2*
	Model	1±0.7	0.7±0.4	1.5±0.5	1.5±0.6	1.3±1	1±0.4
RBC, $\times 10^{12}/L$	Original	4.74±0.57	4.74±0.51	4.53±0.16	4.78±0.42	4.55±0.52	4.17±0.35*
	Model	4.17±0.53	4.9±0.37	4.22±0.41	3.57±1.56	3.19±1.03	3.41±0.27
Hb, g/L	Original	145±18	145±15	134±11	146±14	142±28	120±19
	Model	126±18	150±8	128±19	110±51	100±37	97±6
MCV, fL	Original	84.9±2.6	84.6±2.4	83.2±2.7	82.2±2.3	85.9±4.1	87.5±4.8
	Model	84.3±2.5	81.7±3.1	82.3±2.9	82.7±2.6	86±4.3	86.3±4.4
PLT, $\times 10^9/L$	Original	207±50*	201±69*	189±78*	163±27*	150±21*	160±8*
	Model	24±8	12±5	34±14	31±8	22±7	13±10
PCT, %	Original	0.2±0.03*	0.19±0.04*	0.18±0.06*	0.15±0.02*	0.15±0.01*	0.18±0.02*
	Model	0.02±0.01	0.01±0.01	0.03±0.02	0.03±0.01	0.02±0.01	0.01±0.01
MPV, fL	Original	9.7±1	9.9±1.1	9.9±1.3	10.7±1.4	10.1±0.7	11±1.1
	Model	8.9±1.1	10.3±1.8	9.6±1.2	9.7±1.2	9.3±0.4	10.2±0.8
PDW, %	Original	11.2±2.2	11.5±2.5	11.4±2.1	11.2±3.3	11.7±1.1	14.3±2.9
	Model	10±3.5	9.5±1.7	12.2±2.9	12.7±4.2	9.9±0.7	10.5±0.5
R, min	Original	8.9±1.2*	8.9±1.4*	9.2±0.8*	7.9±2.8*	8.6±0.7*	6.6±3.3
	Model	5±1.9	4.9±0.3	5.4±0.8	4.4±0.4	4.6±0.3	4.8±0.3
K, min	Original	2.3±0.6*	2.3±0.4*	2±0.2*	2±0.9	2±0.4*	1.7±0.3*
	Model	6.8±3.3	9.9±2.7	7.5±2.2	5.5±3.1	7.1±0.1	6±1.7
α , dag	Original	72.3±10.8	66±4.1	68.1±1.9	68±8.5	67.7±3.7	71.3±3.7
	Model	65.4±7.3	59.8±5.6	65.8±3.8	67.7±9.9	66.6±4.4	69.8±3.7
MA, mm	Original	61±3.5*	59.1±5.2*	61.3±3.7*	57.8±8.6*	55.1±2.3*	60.5±6.8*
	Model	31.6±4.9	21.4±5.7	29.2±3.9	32.1±2.1	25.5±4.9	30.2±3.6
CI	Original	-2.1±1.7	-2.2±1.3*	-1.9±0.9*	-1.5±3.3*	-2.3±0.7*	0.1±3.1*
	Model	-4.7±3.1	-6.9±2	-5.6±1.9	-3.6±2.4	-4.6±0.6	-4.2±1.2

α = alpha, CI = coagulation indices, Hb = hemoglobin, K = kinetics, MA = maximum amplitude, MCV = mean corpuscular volume, MPV = mean platelet volume, PCT = = plateletcrit, PDW = platelet distribution width, PLT = platelets, R = reaction time, RBC = red blood cell, WBC = white blood cell.

* Comparison between original blood and model, $P < .05$.

Table 4
Correction effects of manual platelets on the in vitro model of thrombocytopenia (n=20, $\bar{x} \pm SD$) test item.

Test item	Group	1 d	3 d	5 d	7 d	10 d	14 d	p1 (5)	p2 (7)	p3 (10)	p4 (14)
WBC, $\times 10^9/L$	Model	1 \pm 0.7	0.7 \pm 0.4	1.5 \pm 0.5	1.5 \pm 0.6	1.3 \pm 1	1 \pm 0.4				
	PLT-4	0.8 \pm 0.5	0.4 \pm 0.2	1.2 \pm 0.4	1.3 \pm 0.1	1.4 \pm 0.9	0.9 \pm 0.4	0.002	0.001	0.001	0.001
	PLT-22	0.8 \pm 0.5	0.5 \pm 0.2	1.3 \pm 0.5				0.001			
RBC, $\times 10^{12}/L$	model	4.17 \pm 0.53	4.9 \pm 0.37	4.22 \pm 0.41	3.57 \pm 1.56	3.19 \pm 1.03	3.41 \pm 0.27				
	PLT-4	4.07 \pm 0.36	3.92 \pm 0.77	3.72 \pm 0.09	3.73 \pm 0.47	2.49 \pm 0.48	3.3 \pm 0.23	0.395	0.455	0.000	0.000
	PLT-22	4.3 \pm 0.38	3.74 \pm 0.36	3.57 \pm 0.24				0.001			
Hb, g/L	model	126 \pm 18	150 \pm 8	128 \pm 19	110 \pm 51	100 \pm 37	97 \pm 6				
	PLT-4	127 \pm 14	123 \pm 27	111 \pm 7	116 \pm 18	78 \pm 19 [†]	94 \pm 8	0.246	0.361	0.000	0.000
	PLT-22	135 \pm 14	116 \pm 12	108 \pm 11				0.001			
MCV, fL	model	84.3 \pm 2.5	81.7 \pm 3.1	82.3 \pm 2.9	82.7 \pm 2.6	86 \pm 4.3	86.3 \pm 4.4				
	PLT-4	83.8 \pm 2.4	83.2 \pm 2.2	82.9 \pm 3	83.4 \pm 2.3	86.8 \pm 4.1	87.7 \pm 3.8	0.746	0.886	0.058	0.007
	PLT-22	83.3 \pm 2.1	83.4 \pm 2.5	84.3 \pm 2.6				0.665			
PLTs, $\times 10^9/L$	model	24 \pm 8	12 \pm 5	34 \pm 14	31 \pm 8	22 \pm 7	13 \pm 10				
	PLT-4	149 \pm 42	130 \pm 45	150 \pm 34	137 \pm 31	164 \pm 49	99 \pm 19	0.550	0.690	0.515	0.027
	PLT-22	123 \pm 35	127 \pm 30	142 \pm 33				0.490			
PCT, %	model	0.02 \pm 0.01	0.01 \pm 0.01	0.03 \pm 0.02	0.03 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01				
	PLT-4	0.13 \pm 0.03	0.12 \pm 0.04	0.13 \pm 0.03	0.13 \pm 0.03	0.15 \pm 0.04	0.19 \pm 0.29	0.586	0.746	0.427	0.836
	PLT-22	0.1 \pm 0.02	0.11 \pm 0.02	0.14 \pm 0.03				0.017			
MPV, fL	model	8.9 \pm 1.1	10.3 \pm 1.8	9.6 \pm 1.2	9.7 \pm 1.2	9.3 \pm 0.4	10.2 \pm 0.8				
	PLT-4	9.1 \pm 0.4	9.1 \pm 0.4	9.1 \pm 0.4	9.2 \pm 0.5	9 \pm 0.4	9.5 \pm 0.3	0.950	0.916	0.921	0.268
	PLT-22	8.4 \pm 0.5	8.1 \pm 3	10.0 \pm 0.5				0.258			
PDW, %	model	10 \pm 3.5	9.5 \pm 1.7	12.2 \pm 2.9	12.7 \pm 4.2	9.9 \pm 0.7	10.5 \pm 0.5				
	PLT-4	10 \pm 0.8*	9.8 \pm 0.8	9.7 \pm 0.9*	9.9 \pm 0.8 [†]	9.7 \pm 0.9 [†]	9.8 \pm 0.5 [†]	0.817	0.930	0.970	0.988
	PLT-22	8.9 \pm 0.9	9.9 \pm 1.1	12.1 \pm 1.5				0.000			
R, min	model	5 \pm 1.9	4.9 \pm 0.3	5.4 \pm 0.8	4.4 \pm 0.4	4.6 \pm 0.3	4.8 \pm 0.3				
	PLT-4	4.9 \pm 1.4	5.1 \pm 0.4	4.9 \pm 0.6	4.8 \pm 0.8	4.9 \pm 0.6	3.7 \pm 0.5	0.903	0.941	0.977	0.011
	PLT-22	5.1 \pm 1.6	4.5 \pm 0.4	4.5 \pm 0.4				0.423			
K, min	model	6.8 \pm 3.3	9.9 \pm 2.7	7.5 \pm 2.2	5.5 \pm 3.1	7.1 \pm 0.1	6 \pm 1.7				
	PLT-4	1.6 \pm 0.4	1.9 \pm 0.3	2.1 \pm 0.4	2 \pm 0.5	2.3 \pm 0.6	2.1 \pm 0.3	0.485	0.584	0.376	0.411
	PLT-22	1.7 \pm 0.4	1.8 \pm 0.4	1.7 \pm 0.3				0.657			
α , dag	model	65.4 \pm 7.3	59.8 \pm 5.6	65.8 \pm 3.8	67.7 \pm 9.9	66.6 \pm 4.4	69.8 \pm 3.7				
	PLT-4	72.7 \pm 3.6	71.2 \pm 2.1	74.2 \pm 1.4	71.2 \pm 4.8	65.5 \pm 13.1 [†]	75.2 \pm 1.6	0.083	0.217	0.106	0.038
	PLT-22	72.3 \pm 4	73.3 \pm 2.8	74.9 \pm 1.9				0.265			
MA, mm	model	31.6 \pm 4.9	21.4 \pm 5.7	29.2 \pm 3.9	32.1 \pm 2.1	25.5 \pm 4.9	30.2 \pm 3.6				
	PLT-4	57.5 \pm 2.6	54.1 \pm 2.9	54.4 \pm 6.2	51.9 \pm 5.4	49.1 \pm 6	50.6 \pm 2.8	0.219	0.123	0.020	0.010
	PLT-22	53.9 \pm 4.1	52.7 \pm 4.6	50.5 \pm 4.4				0.310			
CI	model	-4.7 \pm 3.1	-6.9 \pm 2	-5.6 \pm 1.9	-3.6 \pm 2.4	-4.6 \pm 0.6	-4.2 \pm 1.2				
	PLT-4	1 \pm 1.2	0.3 \pm 0.7	0.7 \pm 1	0.1 \pm 1.5	-0.6 \pm 1.2	0.9 \pm 0.6	0.338	0.379	0.075	0.042
	PLT-22	0.4 \pm 1.9	0.6 \pm 1	0.5 \pm 0.6				0.937			

α = alpha, CI = coagulation indic, Hb = hemoglobin, K = kinetics, MA = maximum amplitude, MCV = mean corpuscular volume, MPV = mean platelet volume, PCT = plateletcrit, PDW = platelet distribution width, PLT = platelets, R = reaction time, RBC = red blood cell, WBC = white blood cell.

* The comparison between 2 different storage conditions given the same storage time ($P < .05$).

[†] PLT-22 stored at 5 d was compared with PLT-4 at 7, 10, 14 d, respectively ($P < .05$). P1 (5) at 4°C was testified by repeated measurement from storage 1 to 5 d, and P1 (5) at 22°C was also testified by repeated measurement from storage 1 to 5 d. P2 (7) at 4°C was testified by repeated measurement from storage 1 to 7 d. P3 (10) at 4°C was testified by repeated measurement from storage 1 to 10 d. P4 (14) at 4°C was testified by repeated measurement from storage 1 to 14 d.

effects, with no requirement for PLTs to remain in the body beyond 7 to 9 days. Therefore, we felt that the clinical PLT preparations could be classified according to the method of PLT preservation:

- (1) PLTs stored at 22°C that can be used for treatment and prevention, and
- (2) PLTs stored at 4°C, which can be used for emergency hemostasis.

This study only involved in vitro experiments, and employed manually prepared PLT instead of PLT collected via machines. Moreover, given the complexity of in vivo massive blood loss and thrombocytopenia, the findings of this study are yet to be verified by in vivo studies.

In conclusion, PLTs stored for 10 to 14 days at 4°C showed satisfactory correction efficiency for massive blood loss, and may

be recommended for use in the clinical setting for the emergency treatment of massive blood loss.

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