Storage temperature determines platelet GPVI levels and function in mice and humans

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Platelets are currently stored at room temperature before transfusion to maximize circulation time. This approach has numerous downsides, including limited storage duration, bacterial growth risk, and increased costs. Cold storage could alleviate these problems. However, the functional consequences of cold exposure for platelets are poorly understood. In the present study, we compared the function of cold-stored platelets (CSP) with that of room temperature–stored platelets (RSP) in vitro, in vivo, and posttransfusion. CSP formed larger aggregates under in vitro shear while generating similar contractile forces compared with RSP. We found significantly reduced glycoprotein VI (GPVI) levels after cold exposure of 5 to 7 days. After transfusion into humans, CSP were mostly equivalent to RSP; however, their rate of aggregation in response to the GPVI agonist collagen was significantly lower. In a mouse model of platelet transfusion, we found a significantly lower response rate to the GPVI-dependent agonist convulxin and significantly lower GPVI levels on the surface of transfused platelets after cold storage. In summary, our data support an immediate but short-lived benefit of cold storage and highlight the need for thorough investigations of CSP. This trial was registered at www.clinicaltrials.gov as #NCT03787927.

Introduction

After transfusion into humans, CSP were mostly equivalent to RSP yet aggregated significantly less to the GPVI agonist collagen. Standard platelet storage is limited to 5 to 7 days at room temperature (RT; 20-24°C) to limit the risk of sepsis.¹,² As a consequence, they require additional labor and costs resulting from bacterial testing,³ logistical issues with maintaining an adequate supply, and shortages. After transfusion into humans, CSP were mostly equivalent to RSP; however, their rate of aggregation in response to the GPVI agonist collagen was significantly lower. In a mouse model of platelet transfusion, we found a significantly lower response rate to the GPVI-dependent agonist convulxin and significantly lower GPVI levels on the surface of transfused platelets after cold storage. In summary, our data support an immediate but short-lived benefit of cold storage and highlight the need for thorough investigations of CSP. This trial was registered at www.clinicaltrials.gov as #NCT03787927.
that occurs upon transfusion into humans. This dilution factor likely renders the concomitant plasma irrelevant for stored platelet function in vivo. Cold exposure also elicits specific changes in the platelet cytoskeleton, such as actin filament barbed-end capping, actin polymerization, and de novo nucleation. Increased cytosolic calcium and microtubule disintegration accompany the cytoskeletal changes.

Combined, these phenomena mimic responses to agonists and could prime cold-exposed platelets for immediate activation at sites of vascular injury. However, persistent preactivation could also cause desensitization and loss of efficacy. Therefore, it is unclear whether CSP can contribute to thrombus contraction under physiologic flow or generate forces on a single-cell level. Previous studies have examined platelet clot retraction in plasma and whole blood, making it challenging to dissect individual platelet and plasma protein contributions. Few studies have investigated CSP function after transfusion into humans. These studies were predominantly published in the 1970s, and the data are inconclusive.

Given the unclear effect transfusion has on stored platelets and the limited postransfusion human data available, further in vivo investigation of RSP and CSP is urgently needed. If CSP prove effective, a practical application is implementing a dual blood bank inventory, freeing up RSP for prophylactic transfusions and allocating CSP for therapeutic transfusions in actively bleeding patients. Whether CSP have a role in prophylactic transfusions requires additional basic and clinical investigations.

This study addresses the effects of different storage temperatures in state-of-the-art assays while controlling extracellular testing conditions. Furthermore, we evaluate the function of RSP and CSP after transfusion into humans in dual antplatelet therapy and in untreated mice. Our findings highlight the need for posttransfusion and in vivo data on this product.

**Methods**

**Platelet block and post assay**

We collected whole blood and removed platelets by centrifugation. Platelet-poor plasma derived from fresh whole blood was used to dilute the red blood cell–rich sample fraction to yield thrombocytopenic blood. Apheresis platelet-rich plasma (PRP; fresh, RT stored, and cold stored) was added to yield a final target hematocrit of 40% and platelet count of $3 \times 10^{11}$/L.

Reconstituted blood was perfused through a microfluidic channel made with polydimethylsiloxane (PDMS; Sylgard 184) with embedded microscale blocks and microposts (Figure 2A) to monitor platelet aggregation and their production of contractile forces.

Before testing, the microchannels were incubated with rat tail collagen type 1 (200 μg/mL; BD Bioscience) in 0.1 M of acetic acid to support the adhesion and activation of platelets. For testing, a blood sample was flowed through the microfluidic device at a shear rate of 16 000 s$^{-1}$ using a syringe pump (Harvard Apparatus). Platelets within the blood sample attached initially to the blocks and then aggregated to form platelet-rich plugs that bridged the 9-μm gap with each post. After 15 seconds of flow, the shear rate decreased to 500 s$^{-1}$ to reduce the aggregation rate and avoid occluding the microchannel with platelets.

The size of the aggregates and deflection of the post as a result of platelet forces were recorded every 2 seconds over 5 minutes using phase and fluorescent timelapse images obtained on a Nikon Eclipse Ti-E inverted microscope with a 40× objective. The images were then quantified postexperiment using custom MATLAB scripts. The force that aggregated platelets produced was calculated using Hooke's law (data supplement). On day 5 of storage, this protocol was repeated with fresh thrombocytopenic whole blood derived from the same donor and PRP from apheresis platelets stored at room temperature or 4°C.

**Single-platelet contractile force assay**

We used a microcontact-printed, reference-free traction force microscopy approach (Kevin M. Beusmann and M.Y.M., manuscript in preparation). Specifically, a PDMS stamp for microcontact printing was created by casting Sylgard 184 (prepared at a 10:1 ratio) against a silicon master with an array of circular features arranged in an orthogonal lattice. A solution of 2.5 μg/mL of Alexa-Fluor 594–conjugated bovine serum albumin (BSA) was pipetted onto the surface of the PDMS stamps and allowed to adsorb for 1 hour. The stamp was then brought into contact with a polyvinyl alcohol film to transfer the fluorescent bovine serum albumin pattern onto the film. Subsequently, the polyvinyl alcohol film was applied to the surface of a PDMS substrate made with 95% Sylgard 527 (prepared at a 1:1 ratio) and 5% Sylgard 184 (prepared at a 10:1 ratio), which produced substrates with a stiffness of 12 kPa.

The process of microcontact printing resulted in a flexible substrate with a contiguous fluorescent coating containing an orthogonal array of circular regions that were 1 μm in diameter and 2 μm in spacing and lacked fluorescence (termed black dots). The substrates with black dots were treated with 5 μg/mL of von Willebrand factor (VWF; Haematological Technologies) or 5 mg/mL of fibrinogen (Sigma Aldrich) for 1 hour at RT to promote platelet adhesion.

Platelets were obtained, stored, and washed (described in detail in the data supplement). Platelets were further diluted in Tyrode’s buffer and seeded at 2.5 \times 10^{10}/L platelets per mL onto VWF-coated black dots and at 5 \times 10^{10}/L platelets per mL onto fibrinogen-coated black dots to ensure there was separation between the platelets. After 10 minutes of incubation to allow for platelet adhesion, the black dots were gently rinsed with phosphate-buffered saline to remove the unbound platelets. The substrates were then submerged into fresh Tyrode’s buffer for 30 minutes to allow the platelets to spread and generate traction forces. Platelets on the black dots were fixed with 4% paraformaldehyde for 20 minutes and permeabilized with 0.1% Triton X-100 for 20 minutes. Platelet glycoprotein Ib (GPIb) was labeled with a CD42b monoclonal antibody, clone SZ2 (Life Technologies), and a goat anti-mouse immunoglobulin G secondary antibody (Life Technologies). Platelet F-actin was labeled with phalloidin (Life Technologies). Black dots were mounted on glass coverslips using Fluoromount-G mounting medium (Invitrogen) and imaged with confocal microscopy using a Nikon A1R confocal microscope with a 60× oil objective. Single-platelet traction forces were calculated from black dot displacement using regularized Fourier transform traction cytometry. Single-platelet forces were measured in 6 donors treated with VWF and 5 donors treated with fibrinogen. For each donor, each black dot treatment (VWF and fibrinogen), and each condition (fresh, cold stored, and RT stored), an average of 88 platelets were measured for a total of 2905 platelets included in this study.

**Healthy human participant research**

The Western Institutional Review Board–Copernicus Institutional Review Board Group approved our research, and all human
participants provided written informed consent. We conducted the study following the Declaration of Helsinki. All authors had access to primary clinical trial data.

**Healthy human participant demographics and recruitment**

Fourteen participants were enrolled in the study. Six had to end their participation early for different reasons (supplemental Figure 1). Eight had evaluable data for platelet transfusions. One participant did not complete the RT storage arm because of quality control failure (supplemental Figure 1). Overall, the mean age was 28 years (interquartile range, 24-32 years), 2 participants were female (25%), the mean height was 173 cm (standard deviation [SD], ±9.3 cm), the mean weight was 70.6 kg (SD, ±4.7 kg), and the mean body mass index was 23.8 kg/m² (SD, ±2.6 kg/m²).

**In vivo survival and function of transfused mouse platelets**

We collected green fluorescent protein (GFP)-positive whole blood from UbiC-GFP mice in acid citrate dextrose solution A via retroorbital bleeding. PRP was isolated from whole blood and stored at RT or 4°C for 24 hours before transfusion. After platelet
concentrations were adjusted to $2 \times 10^{11}/L$ platelets per mL, we transfused GFP$^+$ platelets into wild-type recipients via tail-vein injection. After transfusion, blood samples were collected from each recipient at 4 and 24 hours. These samples were gated for GFP$^+$ platelets. The transfused platelet population was identified as GFP$^+$ and the endogenous population as GFP$^-$. GPVI levels were evaluated by JAQ-1 antibody binding with an immunoglobulin G–phycoerythrin secondary antibody. The samples were analyzed for activation of mouse αIIbβ3 using Jon/A-phycoerythrin antibody to measure the activation level. We acquired all posttransfusion recovery data, and functional test data were obtained by flow cytometry.

Statistical analysis

Results are reported as mean ± standard error of the mean. Statistical significance was assessed by either paired 2-tailed Student t test or analysis of variance with Tukey correction, as appropriate. A $P$ value
.05 was considered significant. The analyses were performed with Prism (GraphPad, La Jolla, CA).

Results

Poststorage platelet in vitro function

Exposure of platelets to cold induces actin assembly and initiates shape change, resembling the activation response to agonists like thrombin, adenosine 5'-diphosphate (ADP), and collagen. To test if these changes affect thrombus formation and clot retraction in flowing blood, we used a microfluidic collagen-coated block and post assay to quantify aggregation and contractile force generation within a growing thrombus (Figure 1A; supplemental Videos 1-3). In this assay, platelets in a small sample of blood are stimulated to attach and aggregate to form a platelet-rich plug by a local gradient in the shear rate caused by the block and post. Within a few seconds, a plug is typically large enough to bridge the gap between the block and post, and thus, the contractile force produced by the platelets can be measured by how far the post is deflected toward the block.

We stored platelets collected by apheresis for 5 days at RT, as per current clinical practice, or at 4°C, as previously described, and added them to freshly drawn red blood cells and plasma to reconstitute a whole-blood sample. CSP reconstituted in fresh platelet–depleted blood samples adhered readily to the block and post and generated contractile forces that were statistically comparable to those of samples with freshly drawn PRP or RSP (Figure 1B-E). CSP formed significantly larger aggregates than RSP at the early time point (60 seconds; Figure 1B,F-G). However, after 300 seconds, the size of the CSP aggregates decreased, and they were statistically similar to RSP. RSP samples formed consistently smaller aggregates over time than those from fresh blood samples, and the difference between RSP and fresh samples was significant at 300 seconds (Figure 1H). CSP formed larger aggregates.
after 60 seconds, but this did not increase force generation, suggesting individual CSP were generating lower forces. To further evaluate this possibility, we used a novel single-platelet force assay that measures single-platelet contractile traction forces by quantifying the deformation of dots in a fluorescent array coated with either VWF or fibrinogen (Figure 2A). Similar to what we observed in measuring force generation by aggregates formed under flow, individual platelets treated with VWF or fibrinogen were statistically similar among freshly drawn platelets, RSP, and CSP (Figure 2B-C,E-F). These results indicate that the contractile function of platelets might be independent of the storage condition. We noted that traction forces for freshly drawn platelets, RSP, and CSP were more than twofold higher with VWF coating vs fibrinogen coating. On the basis of these results, we concluded that CSP have a higher propensity for shear-induced aggregation than RSP, and CSP can generate contractile forces similar to those of freshly drawn platelets and RSP.

Because we observed larger aggregates under flow with a collagen-coated block and post, we tested the ability of CSP and RSP to aggregate in response to collagen. We observed a trend toward increased aggregation when CSP in concomitant
plasma were stimulated with collagen (Figure 2A), in agreement with previously published data.\(^6,28,29\) However, testing platelets in their stored plasma disregards the large degree of dilution that platelets undergo upon transfusion into the entire blood volume of the recipient. Surprisingly, when testing washed platelets stimulated with collagen, the trend toward better aggregation with CSP compared with RSP was reversed, and there was a clear trend toward more aggregation with RSP (Figure 3B). This reversal was not seen when we stimulated platelets with ADP and arachidonic acid (Figure 3A-B). Collagen is known to activate platelets mainly through GPVI and \(\alpha_{IIb}\beta_3\) integrin activation in whole blood 4 and 24 hours after PLT transfusion with either 24-hour RT- (red circles) or 4°C-stored (blue squares) PLTs after stimulation with 100 nM of convulxin (n = 9; shown as mean fluorescence intensity [MFI] ± standard error of the mean [SEM] of J01/A antibody binding). \(P = .032\) (4 hours) (C) and \(P = .038\) (4 hours) (D), \(\ast\ast P = .001\) (4 hours: RT vs 4°C) (B), \(P = .012\) (24 hours) (C), and \(P = .008\) (24 hours) (D). CVX, convulxin; RBC, red blood cell; WT, wild type.

**FIGURE 4.** Stored platelet (PLT) posttransfusion function in mice. (A) Outline of the mouse PLT transfusion model (details provided in “Methods”). (B) PLT in vivo survival after storage for 24 hours at either RT (red circles) or 4°C (blue squares; n = 9). (C) \(\alpha_{IIb}\beta_3\) integrin activation in whole blood 4 and 24 hours after PLT transfusion with either 24-hour RT- (red circles) or 4°C-stored (blue squares) PLTs after stimulation with 100 nM of convulxin (n = 9; shown as mean fluorescence intensity [MFI] ± standard error of the mean [SEM] of J01/A antibody binding). (D) GPVI expression 4 and 24 hours after transfusion of either 24-hour RT- (red circles) or 4°C-stored (blue squares) PLTs (n = 9; shown as MFI ± SEM of JAAQ-1 with goat anti-rat immunoglobulin G–phycoerythrin secondary antibody). \(P = .032\) (4 hours) (C) and \(P = .038\) (4 hours) (D), \(\ast\ast P = .001\) (4 hours: RT vs 4°C) (B), \(P = .012\) (24 hours) (C), and \(P = .008\) (24 hours) (D). CVX, convulxin; RBC, red blood cell; WT, wild type.

**FIGURE 5.** Stored platelet (PLT) posttransfusion function in mice. (A) Outline of the mouse PLT transfusion model (details provided in “Methods”). (B) PLT in vivo survival after storage for 24 hours at either RT (red circles) or 4°C (blue squares; n = 9). (C) \(\alpha_{IIb}\beta_3\) integrin activation in whole blood 4 and 24 hours after PLT transfusion with either 24-hour RT- (red circles) or 4°C-stored (blue squares) PLTs after stimulation with 100 nM of convulxin (n = 9; shown as mean fluorescence intensity [MFI] ± standard error of the mean [SEM] of J01/A antibody binding). (D) GPVI expression 4 and 24 hours after transfusion of either 24-hour RT- (red circles) or 4°C-stored (blue squares) PLTs (n = 9; shown as MFI ± SEM of JAAQ-1 with goat anti-rat immunoglobulin G–phycoerythrin secondary antibody). \(P = .032\) (4 hours) (C) and \(P = .038\) (4 hours) (D), \(\ast\ast P = .001\) (4 hours: RT vs 4°C) (B), \(P = .012\) (24 hours) (C), and \(P = .008\) (24 hours) (D). CVX, convulxin; RBC, red blood cell; WT, wild type.

CSP function in humans

To test if the loss of GPVI during cold storage led to measurable differences after transfusion in humans, we included 8 healthy human volunteers in a randomized crossover study (described in detail in “Methods”). In brief, we collected apheresis platelets for autologous transfusion. Platelets were stored at either 4°C (CSP) or 22°C (RSP), based on randomization. After 4 days of platelet storage, participants received a loading dose of acetylsalicylic acid (ASA) and clopidogrel and 12 to 24 hours later were transfused the entire unit of stored platelets. We assessed the efficacy of transfusion with platelet function tests at baseline, immediately before transfusion (after antiplatelet drug dosing), and 3 times after transfusion. A washout period of at least 7 days between the first and second rounds ensured clearance of previously transfused platelets and antiplatelet therapy; then, an identical experiment was performed with autologous platelets stored under the alternative condition (supplemental Figure 1; Figure 4A).

**Platelet transfusion parameters**

Platelet counts decreased in all participants after platelet collection (Figure 4B). The absolute platelet counts and the corrected count increments were significantly lower in the CSP transfusion arm after 4 and 24 hours (Figure 4B-C). More information about the transfusion sequence and number of transfused platelets is provided in supplemental Figure 2.
Platelet function testing after transfusion

Because our primary concern was that reduced GPVI levels would lead to reduced response to collagen after transfusion, we isolated and washed platelets from the transfusion recipients. We adjusted the platelet counts and stimulated with collagen. Indeed, we found significantly more aggregation after transfusion of RSP vs CSP, confirming that the loss of GPVI had measurable effects on platelet function after transfusion (Figure 4D). We tested with pathway-specific agonists to gather further information about CSP function in volunteers receiving dual antiplatelet therapy. Arachidonic acid showed an increase in aggregation 1 hour after transfusion of both products compared with post-loading dose levels and a trend toward a reduced aggregation response in the cold-stored group at the 4- and 24-hour time points (Figure 4D). No significant differences between CSP and RSP or evidence of reversal of clopidogrel were seen after stimulation with ADP (Figure 4D; supplemental Figure 3D). To confirm these findings in whole blood, we used the αIIbβ3 integrin activation–based point-of-care assay Verify NOW. One hour after transfusion, we observed reversal of ASA with both CSP and RSP (Figure 4E). After 24 hours, ASA reaction units were significantly lower in the cold-stored group, suggesting the reapparance of ASA inhibition (Figure 4E). We found a trend toward better platelet function after transfusion with RSP in other assays, including a shorter bleeding time at the 1-hour posttransfusion time point, but none of these findings were statistically significant (supplemental Figure 3A-E). Because bleeding time varies among individuals, we normalized the values after the loading dose (Figure 4F) and calculated the change as a percentage for posttransfusion time points. A majority of participants who received RSP showed improvement or no change. In contrast, 50% of CSP recipients showed further bleeding time prolongation (Figure 4F).

Evaluation of mouse platelet GPVI expression and function after transfusion

Our human data suggest that GPVI levels of stored platelets remain reduced after transfusion, without recovery of function or receptor level normalization. However, the study in humans did not allow us to distinguish between transfused (stored) and endogenous platelets. To investigate this further in a living organism without dual antiplatelet therapy, we used a mouse model to track transfused platelets. We obtained and stored platelets from UBiC-GFP mice that have GFP fused to ubiquitin. After transfusion of GFP+ platelets into WT C57BL6/J mice, we obtained blood samples to assess GFP+ platelets and their survival (Figure 5A-B). We tested platelet function by flow cytometry with an activation-specific antibody to αIIbβ3 after stimulation with the GPVI agonist convulxin. Similar to our findings in humans, the αIIbβ3 integrin activation was lower in CSP than RSP posttransfusion (Figure 5C). Consistent with these findings, GPVI levels of CSP were significantly lower than those of RSP after transfusion (Figure 5D).

Discussion

Our study is the first to identify CSP as force-generating contributors in growing thrombi under flow and on a single-cell level. Using clot retraction assays, other groups have examined the function of CSP and reported contradictory results.6,30–32 Plasma factors like thrombin or fibrin generation likely obfuscated the contribution of platelets in these studies. Also, conventional clot retraction assays do not take adhesion or thrombus growth under flow into account. Interestingly, in our assay, the thrombus size was significantly larger with CSP than with RSP, but we did not observe more force generation. This finding could indicate that cold-induced αIIbβ3 preactivation effectively mediates larger, shear-resistant, fibrinogen-dependent platelet-to-platelet aggregates instead of passive agglutination. However, it is unclear why this was not followed by increased thrombus contraction strength. An advantage of our study is that we tested platelets with minimal storage-related plasma factors and on a single-platelet level. However, the spread of data, especially for fresh platelet and CSP force generation, is remarkable and highlights the need to better understand donor-to-donor variability.

Our crossover study in healthy humans shows that CSP show an immediate ASA-reversing effect after transfusion into healthy humans. Although the impact of RSP lasted >24 hours, the inhibition reappeared 24 hours after CSP transfusion. This reappearance of inhibition with CSP indicates that endogenous platelet production (48 hours after antiplatelet drug dosing) was not sufficient to overcome the effect of dual antiplatelet therapy and therefore also serves as an internal no-transfusion control. We doubt the lower platelet count in the recipient after CSP transfusion per se was responsible for this effect, because we observed this phenomenon in whole blood and washed platelets with adjusted platelet counts. A pilot trial in patients undergoing cardiac surgery did not find significant chest tube output differences between RSP and CSP (both stored in platelet additive solution E [PAS-E] instead of 100% plasma).26 Storing CSP in PAS-E only led to a small and nonsignificant reduction in GPVI levels.33 Taken together with our study results, this may suggest that PAS-E is a preferable storage solution for CSP, although more studies in humans are needed to investigate this further. One previous study tested 100% plasma-stored CSP in healthy human participants receiving aspirin and clopidogrel. The authors stored platelets for a shorter period (72 hours) and used bleeding time as the only read-out.34 Unsurprisingly, and very similar to our absolute bleeding time results, they did not observe any significant differences between CSP and RSP. In our study, 50% of the CSP recipients showed a bleeding time prolongation after transfusion, which could hint at a possible disadvantage of this product. Besides apparent efficacy in overcoming some antiplatelet therapy effects, none of our different posttransfusion assays showed any advantage of CSP relative to RSP at any time point after transfusion. These results contradict our and others’ previously published in vitro results.5–13

In our data, we also demonstrate detrimental effects of cold storage. GPVI receptor levels on CSP were reduced, and we observed diminished responses to GPVI agonists pre- and posttransfusion in humans and mice. Our group and others recently observed more collagen-induced aggregation with CSP than RSP with concomitant plasma. It is unclear how the stored plasma supernatant improves GPVI function in CSP. There are reports that GPVI expression levels vary by approximately ~1.5-fold among healthy human donors.35 GPVI function is preserved even at much lower levels than those observed in our study.35 However, the authors tested fresh and otherwise functional platelets at optimized conditions. The loss of GPVI in our study has to be seen in the platelet storage lesion context. Other unfavorable storage-related factors lead to dysfunctional platelets, and any loss of GPVI is likely exacerbated.36,37 In our human study, the participants’ endogenous platelet population was rendered dysfunctional by dual antiplatelet therapy. Thus, the antiplatelet therapy reduced the ability of the endogenous platelets to overcome the
combined effect of storage lesion and loss of GPVI in transfused platelets. Nevertheless, our findings are relevant, because CSP are intended to be transfused into actively bleeding patient populations with known endogenous platelet defects, like surgery patients after cardiopulmonary bypass and trauma patients.\textsuperscript{37} We were able to reproduce our findings of decreased GPVI-mediated platelet function with CSP in a mouse transfusion model without antithrombocyte therapy using the more potent agonist convulxin to stimulate platelets after transfusion. Beyond αIIbβ3 integrin activation, CSP generated more thrombin than RSP, likely mediated by phosphatidylserine-positive procoagulant CSP and microparticles. When procoagulant CSP interact with vascular injury sites, they could promote coagulation locally. Evidence supporting this mechanism has been reported by other groups.\textsuperscript{8,13} This provides a possible alternative mechanism for how CSP could compensate for platelet receptor deficiencies in actively bleeding patients.

In the contractile force flow assay with a collagen-coated block and post, we did not observe significantly different forces between RSP and CSP, even though we replaced most storage plasma with fresh plasma. The presence of VWF and its ability to adhere to collagen and platelets likely helped to overcome the effect of low GPVI levels on CSP in this assay. Whether the same is true in actively bleeding humans remains to be investigated. This mechanistic difference could explain the lack of force increase in CSP, despite larger aggregates, although this warrants further investigation. It is currently unclear how platelets lose GPVI from their membranes during cold storage. Previous studies have shown that it can be cleaved by ADAM 10/17 or taken up by endocytosis.\textsuperscript{38-40} One study observed cleavage of GPVI during RT storage.\textsuperscript{41} Enzymatic activity is likely reduced at 4°C, making cleavage as the primary mechanism unlikely. In a previous study, we saw more microparticles in CSP than RSP.\textsuperscript{28} This suggests that loss of GPVI by membrane microvesicles may be responsible for the findings in the current study. Although we show GPVI levels are essential for CSP function, we make no claim that loss of GPVI affected platelet clearance. Other groups have identified different CSP clearance mechanisms.\textsuperscript{42-44}

In summary, our study is the first to provide a thorough assessment of the contractile function and the pre- and posttransfusion functions of CSP compared with the current clinical standard. Importantly, clinical trials are required to test the efficacy of CSP in actively bleeding patients.

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Authorship

Contribution: J.M. recruited participants, performed apheresis collections, performed experiments, analyzed data, and helped write the manuscript; S.L.B., A.M.O., M.Y.M., C.U., D.B., J.R.F., L.F., and K.H. performed experiments, analyzed data, and helped write the manuscript; J.C. and B.O. recruited participants, performed apheresis collections, and performed platelet transfusions; Y.W. and Y.S. performed experiments; X.F., J.-F.D., and N.J.S. designed experiments, analyzed data, and helped write the manuscript; and M.S. designed the study, reviewed and analyzed data, and wrote a first draft of the manuscript.

Conflict-of-interest disclosure: N.J.S. is a cofounder and board member of and has equity in Stasys Medical Corporation and is a scientific advisor for and has equity in Nanosurface Biomedical, Inc. M.S. has received research funding from Cerus. The remaining authors declare no competing financial interests.

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