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# Omega-3 Polyunsaturated Fatty Acids EPA and DHA Prevent Platelet GPVI Loss and Extend the Shelf-Life of Cold-Stored Platelets

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## Introduction

- Room temperature (20–24°C)-stored platelets (RTPs) are currently used clinically due to longer post-transfusion circulation time. However, RT-stored platelets are generally limited to a 5-day shelf-life due to high risks of bacterial proliferation.
- While Cold-Stored Platelets (CSPs; stored at 4°C) offer superior hemostatic function and a significantly lower risk of bacterial contamination compared to RTPs, their clinical utility is severely limited by rapid clearance from circulation. Because they are cleared by the liver within hours of transfusion, they are unsuitable for prophylactic use and are strictly optimized for immediate, therapeutic clotting in active hemorrhage.
- Acting as the primary receptor for collagen, glycoprotein VI (GPVI) mediates platelet adhesion and aggregation upon vascular injury. Previous studies showed GPVI could be a valid marker of platelet storage lesion, and significantly reduced GPVI levels were observed after cold storage of 5-7 days.

## Hypothesis

Omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) will improve cold-stored platelet functionality through preventing apoptosis, stabilizing membrane proteins, and protecting membrane glycoproteins such as GPVI during storage.

## Materials and Methods

**Human platelets samples:** Apheresis platelet-rich plasma (PRP) units were obtained from healthy donors. All units were stored at 4°C until testing on days 1, 3, 7, 14, and 21.

**Optical Aggregometry Assay:** Platelet aggregation was measured using optical aggregometry on a Chrono-Log 700 aggregometer to assess the efficacy of the omega-3 fatty acids in platelet function recovery from cold storage lesion. A combination of ADP, collagen, and epinephrine (5 µM adenosine diphosphate (ADP) + 1 µg/mL collagen + 2 µM EPI) was used as a combined agonist for each sample. Agonist response was recorded for 6 minutes according to manufacturer's recommendations, and the maximum amplitude of response to agonists was reported.

**Flow Cytometry Assay:** GPVI on the surface of platelets was assayed as an indicator of platelet cold storage lesion. As an important adhesive receptor, GPVI contributes significantly to thrombus formation. Platelet samples were assessed for various activation, apoptosis, and constitutive markers using flow cytometry. Phosphatidylserine (PS) is a marker on the surface of cells that is a key indicator of platelets undergoing apoptosis.

**Adenosine Triphosphate Assay:** Adenosine triphosphate (ATP) was measured via luminescence analysis as an indicator of platelet viability.

## Results

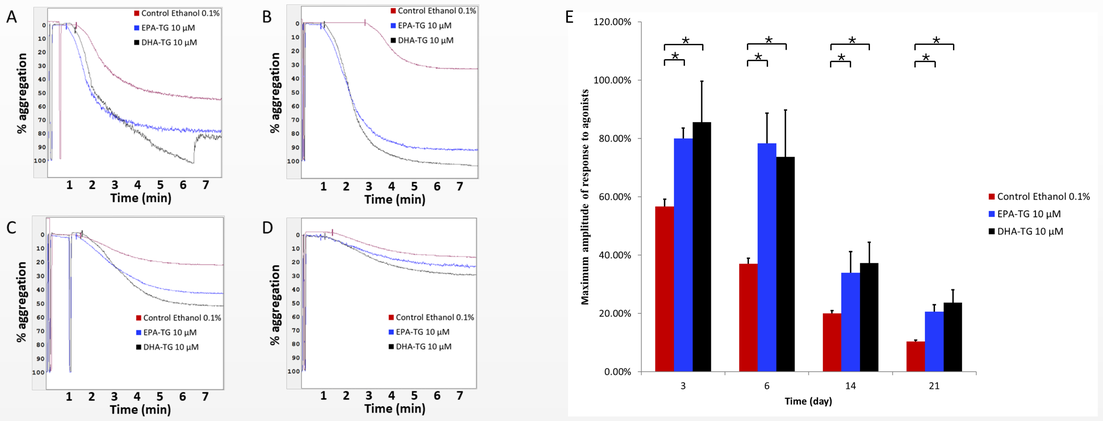


**Figure 1. Treatment with EPA-TG and DHA-TG improved human platelet aggregation function.** Platelet aggregometry induced by agonists collagen, ADP, and epinephrine were performed for the assessment of 1,2,3-Trieicosapentaenoyl Glycerol (EPA-TG) and 1,2,3-Tridocosahexaenoyl Glycerol (DHA-TG) on human platelet function during cold storage according to the manufacturer's guidelines. The maximum amplitude of response to agonists at (A) day 3, (B) day 6, (C) day 14, and (D) day 21 of cold storage are shown as the blue line for PRP treated with EPA-TG 10.4 µM, the black line for PRP treated with DHA-TG 10.4 µM, and the red line for PRP treated with control Ethanol 0.1%. (E) Maximum amplitude following agonist treatment shown as mean ± SD (n = 3 for each group). * means $P < 0.05$.

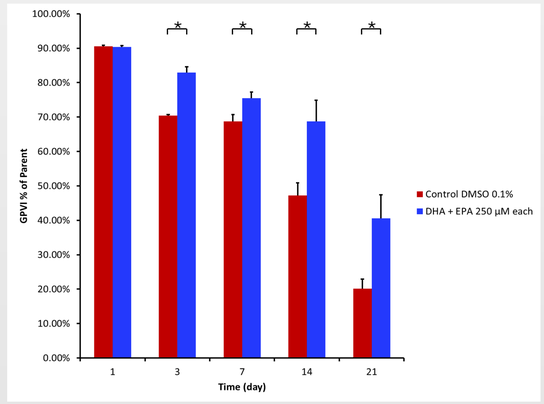


**Figure 2. Treatment with EPA and DHA in cold-stored platelet-rich plasma (PRP) prevented platelet GPVI loss during 21 days of storage period.** GPVI is a crucial collagen receptor on platelets, acting as a primary sensor for damaged vessel walls, triggering platelet activation, aggregation, and thrombus (clot) formation by initiating signaling cascades essential for hemostasis and thrombosis. Data are presented as mean ± SD (n = 5 for each group). * means $P < 0.05$.

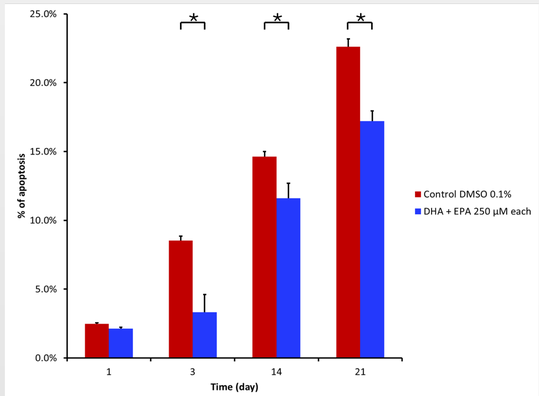


**Figure 3. EPA and DHA inhibited platelet apoptosis in cold-stored platelet-rich plasma (PRP) during 21 days of storage period. "**PS+" refers to a marker that specifically detects the presence of phosphatidylserine (PS), a phospholipid typically exposed on the surface of a cell undergoing apoptosis, making it a key indicator of cell death when analyzed using flow cytometry. Data are presented as mean ± SD (n = 5 for each group). * means $P < 0.05$.

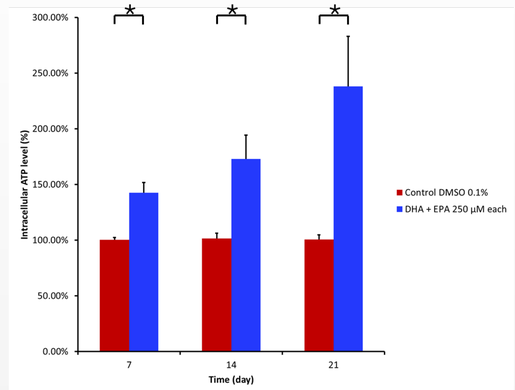


**Figure 4. EPA and DHA treatment increased platelet viability in cold-stored platelets at Days 7, 14, and 21 compared with control.** Adenosine Triphosphate (ATP) Luminescent Cell Viability Assay was performed for quantification of intracellular ATP by luminescence analysis for platelet viability. Data are presented as mean ± SD (n = 3 for each group). * means $P < 0.05$.

## Conclusions

- EPA and DHA treatment prevents platelet GPVI loss and improves platelet function compared to blank control.
- Addition of EPA and DHA significantly increases the viability of cold-stored platelets and inhibits platelet apoptosis from cold storage lesion.
- The aggregation function of day 14 cold-stored platelet with omega-3 PUFAs treatment was the same as the aggregation function of untreated day 3 cold-stored platelets, which suggest that addition of EPA and DHA would extend the shelf-life of cold-stored platelets by more than 10 days.
- Future studies will focus on precision dosing and developing advanced formulations such as lipid-based nanoparticles for maximum outcome.

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