1250
doi:10.1017/S1431927618006736
Microsc. Microanal. 24 (Suppl 1), 2018

Ultrastructural Analysis of Platelets During Storage in Different Buffers

Ru-ching Hsia[1,2], Ramandeep Kaur[2], Rosemary Schuh[3], Andrew Saladino[3,4], N. Jennifer Klinedinst[5], Gary Fiskum[3]

[1.] Electron Microscopy Core Imaging Facility, University of Maryland Baltimore, Baltimore, USA
[2.] Department of Neural & Pain Sciences, University of Maryland School of Dentistry, Baltimore, USA
[3.] Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, USA
[4.] Department of Pathology, University of Maryland School of Medicine, Baltimore, USA
[5.] University of Maryland School of Nursing, Baltimore, USA

Platelets, also called thrombocytes ("blood clot cells"), are a component of blood whose function is to stop bleeding and maintain hemostasis. Platelet concentrates (PCs) are a critical resource in treating patients with bleeding disorders. The quality of PCs is important in its functionality *in vivo* as activated platelets can cause adverse effects to the patient. Currently, PCs are stored in the blood bank, at room temperature and under gentle agitation, for five days before deemed inactive and discarded. In this study, we tested the preservation of PCs stored in different storage buffers at days 0, 3 and 6 by analyzing the ultrastructural changes using transmission electron microscopy (TEM). The ultimate goal of this project is to develop an improved platelet storage buffer to extend platelet shelf life or enhance the quality of preservation.

Platelets were isolated from fresh blood obtained from volunteers, as approved by the University of Maryland, Baltimore Institutional Review Board. Platelets were stored in either ACD-A or PAS storage buffer with or without additive. ACD-A buffer consists of 38 mM citric acid, 74.8 mM sodium citrate tribasic and 123 mM dextrose. PAS buffer consists of 77.3 mM sodium chloride, 32.5 mM sodium acetate, 10.8 mM sodium citrate, 6.7 mM monosodium phosphate and 21.5 mM disodium phosphate. Two different additives, beta hydroxybutyrate (BHB) and sodium pyruvate (PYR) at 30 mM concentration were also tested for their effect on platelet storage. Platelet cells were collected at different times during the storage period, processed, embedded in spurs resin and ultrathin-sectioned by morphometry analyses by TEM. The quality of platelets was determined following the scoring criteria of Neumuller et al [2]. Figure 1 illustrates the criteria for the scoring of platelet degeneration. ImageJ (NIH) [3] software was used to perform platelet counts, identify and mark different stages of necrosis.

Our results indicate that PAS provides better platelet preservation when compared to ACD-A. A higher percentage of normal platelets was observed at day 6 in PAS (Figure 3A). This finding is consistent with a parallel study examining the metabolic activities of platelet cells in storage (not shown). Figure 2 illustrates platelet ultrastructural changes at days 0, 3 and 6 after storage in PAS or ACD-A storage buffer. Furthermore, platelet preservation can be further enhanced by adding PYR or BHB in the PAS storage buffer (Figure 3B).

This study confirms that TEM ultrastructural analysis can be used to evaluate platelet preservation in storage. However, this result needs to be confirmed with a larger sample size. To our knowledge, PAS and ACD-A are used in different blood banks. ACD-A contains higher concentration of sugar than PSA. The hyperglycemic solution can lead to oxidative stress and platelet apoptosis/necrosis. Additionally, our results also indicate that platelet preservation may be improved by adding PYR or BHB in the storage buffer. PYR and BHB have both been shown to enhance mitochondrial

energy metabolism. Further analyses using various concentrations or combinations of additives may be necessary to further optimize platelet preservation in storage.

References:

[1] Sawatzke & Solomons. J Clin Pathol 33(1980), p. 600.
[2] Neumuller et al. Transfus Med Hemother 40 (2013), p.101.
[3] Abramoff et. al. Biophotonics International 11(2004), p. 36.
[4] The authors acknowledge that a major instrument used in this study was funded by NIH Shared Instrument Grant 1S10RR26870-1.

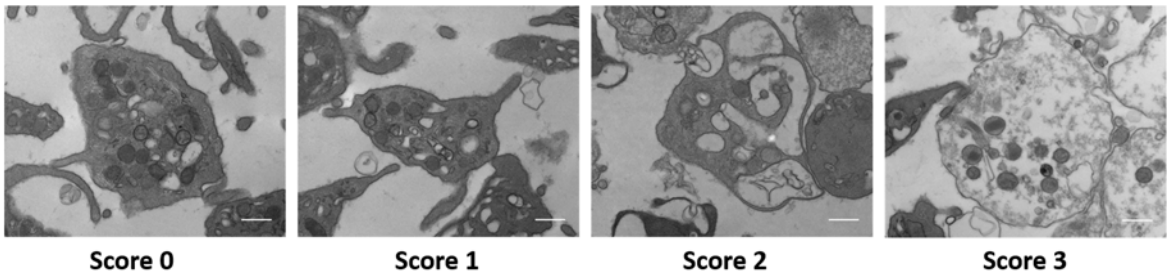

Figure 1. Platelet Scoring Criteria. Score 0: Unchanged discoid form showing peripheral microtubular coil (MTC). Score 1: Formation of filopodia, and dilatation of the open canalicular system (OCS). Score 2: Pronounced shape alterations, centralization of the MTC and processing degranulation. Score 3: Degeneration and necrosis. *Bar* =500 nm.

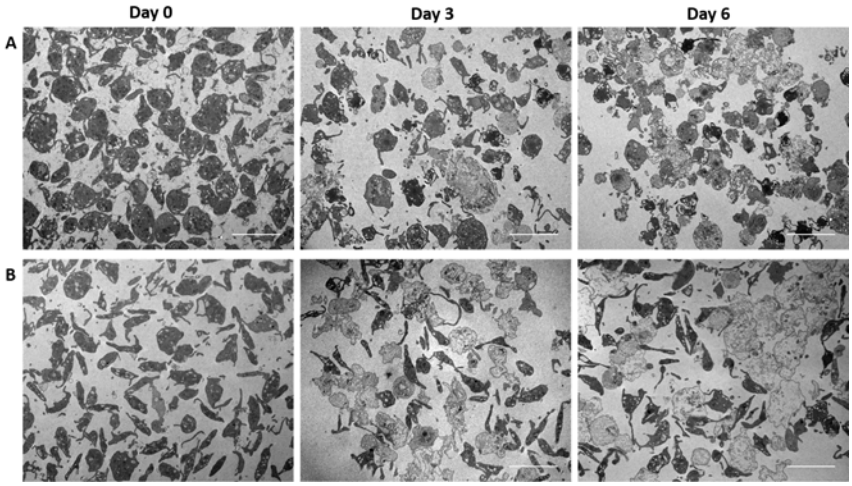

Figure 2. Ultrastructure of platelets in storage buffer. TEM images of platelets stored in ACD-A (A) and PAS (B) buffer from day 0 to 6. *Bar* = 500 μm.

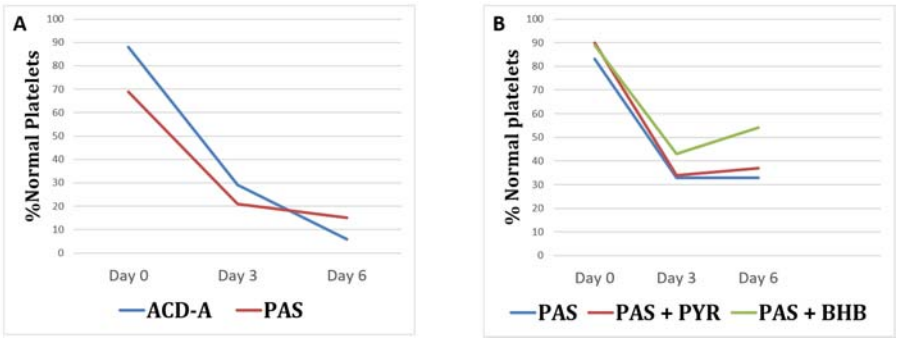

Figure 3. Platelet preservation during storage. Percentage of normal platelets stored in ACD-A and PAS buffers (A). Percentage of normal platelets stored in PAS buffer with addition of 30mM PYR or BHB (B).

https://doi.org/10.1017/S1431927618006736 Published online by Cambridge University Press