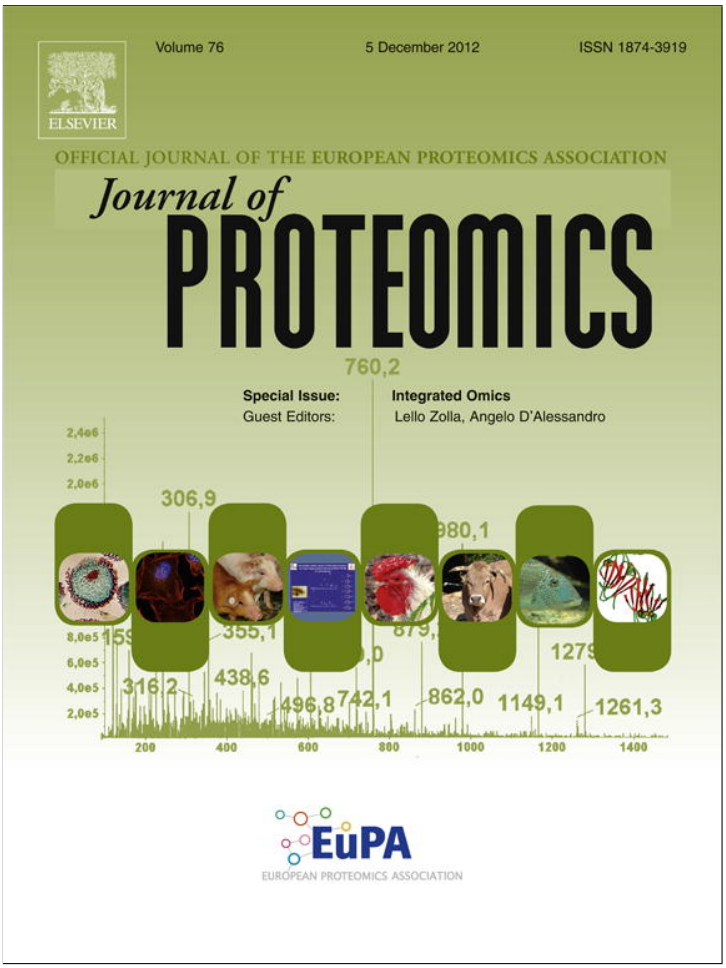

JOURNAL OF PROTEOMICS 76 (2012) 163–167

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Systems biology of stored blood cells: Can it help to extend the expiration date?☆

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ARTICLE INFO

Available online 28 August 2012

Keywords:
Systems biology
Platelet storage lesions
Red blood cell storage lesions

ABSTRACT

With increasingly stringent regulations regarding deferral and elimination of blood donors it will become increasingly important to extend the expiration date of blood components beyond the current allowed storage periods. One reason for the storage time limit for blood components is that platelets and red blood cells develop a condition called storage lesions during their storage in plastic blood containers. Systems biology provides comprehensive bio-chemical descriptions of organisms through quantitative measurements and data integration in mathematical models. The biological knowledge for a target organism can be translated in a mathematical format and used to compute physiological properties. The use of systems biology represents a concrete solution in the study of blood cell storage lesions, and it may open up new avenues towards developing better storage methods and better storage media, thereby extending the storage period of blood components.
This article is part of a Special Issue entitled: Integrated omics.

1. Introduction

Since the time of the ancient Greeks, blood has been revered for its healing and regenerative power, so much so that bathing in blood and ingesting it were considered a remedy well into the middle ages [1]. Blood has two abundant cell types, red blood cell (RBC) and platelet (PLT). RBC has the physiological role of transporting oxygen from the lungs to the tissues and carbon dioxide back to the lungs, while PLT participates in homeostasis by forming blood clots and serving as a reservoir of different cytokines and growth factors.

Modern medicine is greatly dependent on the banking and transfusion of blood products for use as support treatments during cancer therapies, for trauma and burn treatments, obstetrics, and various surgeries. Blood components used in these treatments are derived and processed from volunteer blood donors that have undergone a strict screening process.

A major problem that blood banks will face in the near future is a steady decline in the number of qualified blood donors [2]. With increasingly stringent regulations regarding deferral and elimination of blood donors it will become increasingly important to extend the expiration date of blood components beyond the current allowed storage periods. Currently, the storage period ranges from 35 to 42 days (at 2–6 °C) for RBC concentrates and 5–7 days (22 °C with agitation) for PLT concentrates [3]. One reason for the storage time limit for blood components is that PLT and RBC develop a condition called storage lesions during their storage in plastic blood containers.

Red blood cell storage lesion (RSL) has various effects on the RBC, including changes in the metabolic status of the cell that include a breakdown of metabolic sugars to lactate and protons via glycolysis which subsequently result in a lower pH and a loss of 2,3-diphospholgycerate (2,3-DPG). The loss of 2,3-DPG coincides with a shape change and a membrane loss

☆ This article is part of a Special Issue entitled: Integrated omics.

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http://dx.doi.org/10.1016/j.jprot.2012.08.014

in the RBC due to oxidation of proteins and lipids that leads to a breakdown of the RBC and their failure to survive once they return to circulation [3]. RSL can also lead to secondary risks that include an accumulation of potassium in the RBC unit that can increase the risk of arrhythmias upon transfusion.

Platelet storage lesion (PSL) leads to a significant loss of platelet function during 5 days of storage. This lesion causes changes in platelet morphology, physiological activation with subsequent exposure of membrane phosphatidylserine and release of platelet granules. As consequence of these changes, platelets lose the ability to aggregate and show reduction of matrix adhesion properties. Changes in cell metabolism are also observed. In fact platelets seem to experience anaerobic glycolysis resulting in accumulation of lactic acid and decrease in pH, leading to decline in mitochondrial activity and subsequent apoptosis [4].

While the underlying mechanisms of RSL and PSL are far from being fully understood, it is clear that they are caused by a multitude of different components. Development of RSL and PSL is usually related to collection methods, storage methods, and post collection manipulation including storage solutions. A full understanding of storage lesions in red blood cells and platelet is needed in order to figure out ways to extend the storage period of blood components. Current methods that are used to detect storage lesions have their limitations, and therefore new tools are needed in order to decipher the storage lesion of RBC and PLT.

Integrated omics has been an overlooked concept in transfusion medicine and storage of transfusion products. This special issue offers a glimpse into what is happening in this field, and it provides evidence supporting the important role that integrated omics platforms can play in the study of blood cells.

For instance, an integrated omics strategy was used by Thiele et al. to map out the molecular pathways involved in PSL, describing integrin αIIbβ3 and focal adhesion signaling pathways as key players in irreversible PSL and subsets of PLT mRNA that may be important in early PSL formation [5].

Antonelou et al. address the subject of pre-storage leukoreduction of RBC concentrates with focus on senescence and oxidative stress associated with molecular and cellular modifications. Their data indicates that residual leukocytes and platelets that are present in non-leukoreduced RBC concentrates may impose an additional stress on the RBC affecting most RBC removal signaling and thereby their structural and functional integrity [6].

Delobel et al. focused on carbonylation that is believed to be a pinnacle part of oxidative stress of RBC during the storage. Their conclusion is that carbonylation of cytoplasmic and membrane proteins differs during the storage period, describing two spate steps in the evolution of carbonylation during RBC concentrate storage [7].

Vesiculation from RBC is believed to prevent untimely removal of RBC from the circulation by removing damaged membrane parts from the RBC, that are enriched with removal signals such as phosphatidylserine and IgG [8]. Bosman et al. mention the generation of vesicles during blood bank storage [9]. Proteomics analysis focused on the aging and vesiculation of RBC in vivo may point out solutions to prevent or reduce the formation of vesiculation of RBC during blood banking.

This issue is also presenting alternative approaches to ensure more efficient and safer products in transfusion medicine, as reported by Schubert et al., which suggest the use of integrated omics as a tool to predict transfusion outcome and improve donor management, proposing RRap1 and RhoGDI as valuable markers in this context [10].

The increasing use of integrated omics approaches for the study of blood cell storage lesions may be the prelude to integrated systems biology platforms achieved by integration of omics data in mathematical models.

2. Systems biology

Systems biology is suddenly everywhere. Its community includes researchers worldwide, spanning disciplines from metabolic engineering to life sciences. In classical biology, the reductionist approach has been pervasive over the last century. It considers a complex system as the sum of its parts that are described with a hierarchy of organizations. This approach has been successfully used in biology to identify its molecular components and, in part, their biological functions. Unfortunately, it falls short when considering the nature of the links between components and the network resulting from the assembly of all such links.

Systems biology is about putting together rather than taking apart, and it provides comprehensive biochemical descriptions of organisms through quantitative measurements and data integration in mathematical models. The driving force for this emerging field has been the development of high-throughput technologies that generate the so-called omic data sets. In fact, in the last decade, multi-omics high-density data sets have been generated, providing extensive knowledge of the biological components while raising the issue of data curation and integration at the same time.

The biological knowledge for a target organism can be translated in a mathematical format and used to compute physiological properties. This translation results in a genome scale metabolic reconstruction, which represents a biochemical, genetic and genomic (BIGG) knowledge base [11] for a target organism. These reconstructions may then be used to investigate fundamental biological questions, guide industrial strain design, and provide a systems perspective for analysis of the expanding ocean of omics data [12–14].

In 2007 the first genome scale metabolic reconstruction of *Homo sapiens* (Recon 1) was presented [15]. Recon 1 represented the comprehensive knowledge of human metabolism and suddenly it was realized that it could be used as a starting point for generating tissue and condition-specific models by integrating omics data. In fact, metabolomics, proteomics, and transcriptomics data obtained from a particular tissue or cell line in defined conditions can be used to generate a model which is not only specific for the tissue but for the conditions investigated as well. The procedure to obtain such a reconstruction is well defined and partially automated [16], leading to an exponential increase in the number of reconstructions and in silico modeling strategies [17,18].

Data integration with mathematical models and the capability to generate multi-omic datasets are now leading to a new approach where the generation and integration of

data are part of a new platform for integrated systems biology. The fast development of such a platform is desirable and necessary in systems biology and requires multi-disciplinary input from an array of biological, computational, analytical, and clinical experience.

3. Systems biology of stored blood cells

Red blood cells and platelets are enucleated cells with relatively simple metabolism, whose biochemistry and physiology are very well studied. In fact, the first mathematical model of erythrocytes was presented in 1974 and it included only the glycolysis [19]. In the last 10 years, the consolidation of well defined analytical platforms for proteomics has led to an increased number of studies focused on the proteome of platelets and red blood cells, describing cells that are metabolically more active than previously thought.

In 2011, Bordbar et al. used the available proteomics data to reconstruct the most expansive description of RBC metabolism to date, and used it to simulate its physiological and pathophysiological states. Using in silico simulations, the study showed that RBCs can be used as a diagnostic for drug treated conditions [20]. In a previous study, Nishino et al. developed a mathematical model for erythrocytes and used it to predict metabolic changes occurring during their long-term storage. In silico simulations reproduced the reported time-courses of ATP and 2.3-DPG during storage and these predictions were validated by comparison with metabolomics data [21].

While several proteomics studies have also been reported for the study of storage lesions of both platelets and red blood cells, there is a lack of metabolomics studies on this topic to date. Gevi et al. reported a study in which intra- and extra-cellular metabolites of red blood cells were profiled during cold storage. During storage, a continuous exchange of metabolites occurs between the extracellular and intracellular environments, as cells take up nutrients and excrete metabolites. By monitoring the exchange of metabolites between the intracellular and extracellular environments and the intracellular metabolites over time, the authors were able to provide a more detailed picture of the red blood cell metabolism [22]. They confirmed the rapid fall of glycolysis rate and the accumulation of glycolysis end products together with purine salvage pathway products. Moreover they highlighted the role of oxidative stress during the storage. In a successive study, D'Alessandro et al. have used an integrated metabolomics and proteomics approach for a time course investigation on stored RBC, pointing out that most of the biochemical and structural changes associated with the RSL occur after 2 weeks of storage [23].

The increasing availability of omics data for stored blood cells will lead to the development of integrated systems biology platforms. Nevertheless to reach this objective it is necessary to develop and refine procedures, on the computational and experimental sides.

This synergy between experimental and computational sciences may provide further insight into the molecular mechanism of RSL and PSL.

Here, a pipeline for the use of in silico strategies in the study of the blood cell storage lesions is suggested (Fig. 1).

i. Step 1: Context-specific metabolic reconstruction.
The first step consists in generating a cell specific metabolic reconstruction. A global human network reconstruction (Recon 1) has served as an effective starting point to develop tissue specific models. Further refinement of these reconstructions can be achieved by

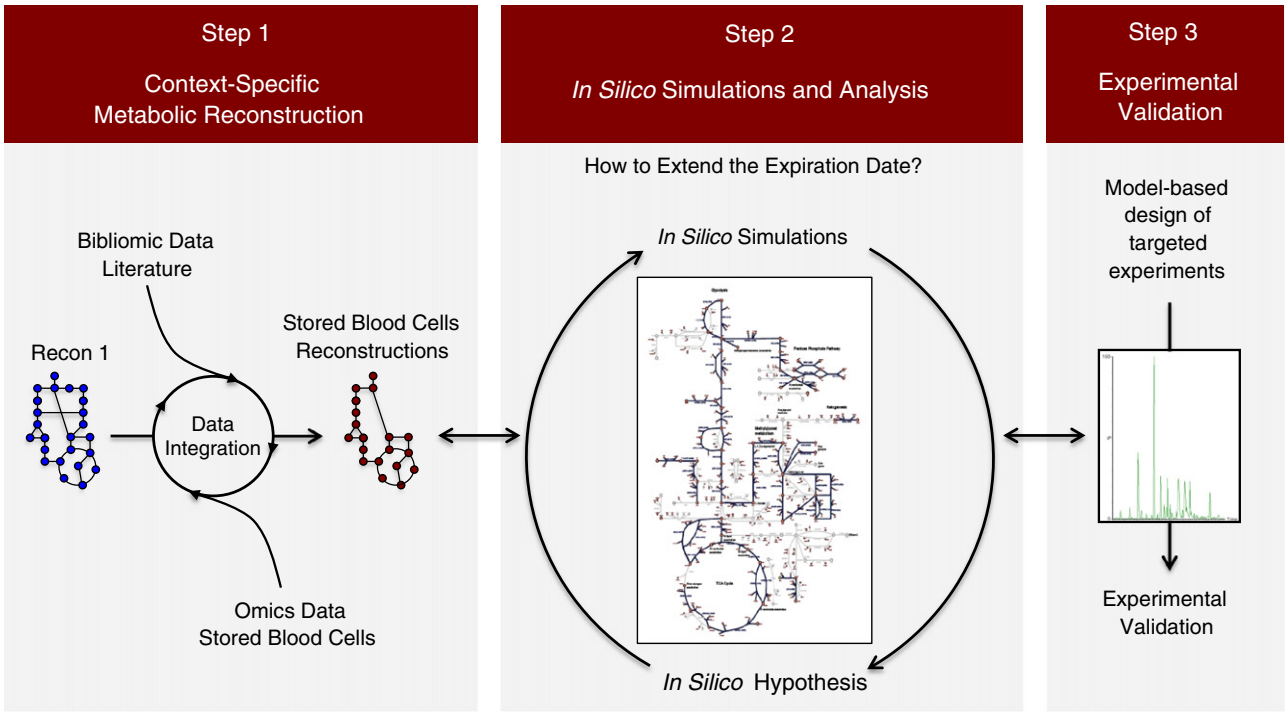

Fig. 1 – Systems biology pipeline to study stored blood cells.

condition specific tailoring of the metabolic network through integration of context-specific omics data by using various algorithmic approaches [24–27].
Regardless of the method however, in order to generate high quality models, some steps will require manual curation and in-depth literature search to ensure high quality and coverage of the reconstruction.
Quantitative omics data focusing on blood cell storage lesions are required in order to achieve these objectives.

ii. Step 2: In silico simulations and analysis.
Once the cell/condition specific model has been generated, it can be used to study blood cell storage lesions. In silico simulations by the use of constraint-based modeling [28] can unravel mechanisms and interactions of cellular networks involved in the development of blood cell storage lesions. There are a plethora of methods that have been developed and will likely continue to be developed that enable in silico analysis [29]. Subsequently, constraint-based modeling can be used to drive experimental hypothesis for extending the expiration date.

iii. Step 3: Experimental validation.
Experimental validation involves a dynamic process referring back to iterative revisions to the model. In silico generated hypotheses are tested and compared with experimental results. In case of erroneous predictions, revision of the model is required and new hypotheses are generated. The number of iterations and rounds of hypothesis testing is not fixed, and can in principle be continued indefinitely, producing more nuanced and detailed levels of understanding of underlying biochemical mechanisms through evaluation of multiple hypotheses.

The use of systems biology and integrated omics represents a concrete solution in the study of blood cell storage lesions, and it may open up new avenues towards developing better storage methods and better storage media, thereby extending the storage period of blood components. This is crucial to overcome the increasing deficit in the supply and rising demand of blood components in the future.

Acknowledgments

This work was supported by the European Research Council grant proposal no. 232816.

The authors would like to thank Neema Jamshidi and Alex Thomas for fruitful discussions and helpful comments.

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