The use of novel biopolymers to mediate red blood cell membrane permeability enabling loading of intracellular protectant and long term stabilisation by freeze-drying

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Abstract

Blood preservation technologies depend upon refrigeration (1), which has a number of drawbacks including the cost and logistics of cold chain management. The shelf life of red blood cells preserved by refrigeration alone is just 35-42 days. Meanwhile medical advances have increased demand for blood and blood products. The preservation of red blood cells (RBCs) by drying involves some tough challenges. Structural integrity must be maintained and the cells must also continue to function as oxygen carriers with no oxidation of haemoglobin.

In this study, we have shown that red blood cells can be successfully freeze dried and rehydrated with minimal degradation. A novel biopolymer was investigated as a possible membrane modifier to enable loading of protective agent into the RBCs prior to freeze-drying by means of a controlled incubation step. A series of excipients and protectants were studied, as well as a range of freeze drying parameters, including cooling rate, temperature and drying time. The success of these trials was assessed using ultraviolet (UV) spectrophotometry at a series of wavelengths to quantify the extent of haemoglobin remaining in RBC pellets versus supernatants after centrifugation, and the extent of haemoglobin oxidation.

In the absence of the novel biopolymer, levels of RBC "survival" of 96% were achieved, suggesting that intracellular protectant may not be necessary to prevent haemolysis during lyophilisation, although oxidation levels of haemoglobin were quite high; typically 60%. While the use of the biopolymer did not necessarily lead to higher RBC survival under the conditions employed here (the maximum survival observed in the presence of the biopolymer was 85%), it was notable that only in samples containing the biopolymer was the extent of haemoglobin oxidation reduced to below detectible levels, and was typically below 10% wherever the biopolymer was included.

Prevention of haemoglobin oxidation may have been attributable to the direct action of the biopolymer itself and/or the presence of intracellular protectant facilitated by the presence of the biopolymer. The mechanism of action of the biopolymer may be similar to that recently proposed by Lynch et al for the cryopreservation of RBCs (2).

This study has shown that red blood cells can be successfully freeze dried and rehydrated with minimal degradation. The lessons learned in this study will be applied to further studies on RBCs and nucleated cells.

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References:

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