Shelf-life enhancement of donor blood by He–Ne laser biostimulation†

Al-Khalid Isam Zuhaier[1,2,*], Kosa Mohammad[2], Sandhanasamy Devanesan[3], Mohamad Saleh AlSalhi[3], Saradh Prasad[3] and Vadivel Masilamani[3]

[1]Vice-Rectorate for Graduate Studies and Research, King Saud University, Riyadh, Kingdom of Saudi Arabia
[2]The Higher Institute of for Laser Research and Applications, Damascus University, Syria
[3]Research Chair in Laser Diagnosis of Cancer, P.O. Box 2455, College of Science, Department of Physics, King Saud University, Riyadh, Kingdom of Saudi Arabia

Shelf life of stored blood in a bank is an important parameter for the effective use of blood drawn from the donors. Several attempts have been made (such as antioxidant treatment and magnetic field intervention) to improve the above lifetime of 42 days. Here, we show that biostimulation by a He–Ne laser could enhance the shelf life to 63 days. The results are based on the fragility and conductivity measurement of red blood cells.

Keywords: Biostimulation, He–Ne laser, shelf life, stored blood.

BLOOD constitutes 7% of the human body weight and is about 5 litres in volume. It is a life-saving fluid in the body and has the most essential function of supplying nutrients and oxygen to all parts of the body. It also removes CO_2 from the system. Blood contains cellular components (red blood cells (RBCs); white blood cells (WBCs); and platelets) and plasma (which contains a host of proteins, hormones, glucose, etc.). There are blood banks all over the world collecting this vital fluid from donors for transfusion into acceptors (during major surgery or in trauma care). The blood samples collected should be stored for a long time for effective use. It is important to note that the blood kept stored between 1–6°C up to a maximum of 42 days only can be administered to any acceptor according to the US federal law. This is because RBC gets spoiled due to slow haemolysis and release of nitric oxide (NO), which leads to some complications in the acceptor[1].

In this line, several efforts have been made to enhance the storage or shelf life of the blood collected. For example, trilazd mesylate (TM), a powerful antioxidant has been used to protect the stored RBCs against oxidative damage of gamma irradiation. It was found to decrease the osmotic fragility (OF), thus improving shelf life[2].

Another interesting aspect is the effect of magnetic field in improving the erythrocyte OF. An improvement by 15% in shelf life has been observed using a magnetic field of 0.15T for 30 min (ref. 3). In the present study, to enhance shelf life we have used low-power laser irradiation instead of chemical or magnetic field interaction.

Low-power laser treatments (LPLTs) have a wide range of applications from diabetic wound-healing to pain removal by acupuncture[4]. Some workers have used a He–Ne laser for photosensitized oxidation of RBCs[5], inhibition of platelet function[6,7], and to obtain biophysical information about blood[8]. Others have tried laser priming of human leucocytes[9] and even laser-induced thermal coagulation of whole blood[10].

In a study[11] using a red He–Ne laser of 800 μW and exposure of 300 sec, RBC deformability has been reduced by 30%. In another study[12], improved ion flow on the RBC membrane has been shown. A comparative study of red and green lasers on rheological factors of human blood was also made[13]. In spite of the variety of works undertaken, to our knowledge there are no reports on the enhancement of shelf life for stored blood. In the present study we show 60% enhancement in the shelf life of blood by LPLT of whole blood. The only comparable report is the enhancement of shelf life of blood by 50% by deoxygenation using chemical methods[14].

Fresh blood (5 ml) was taken from the blood bank of King Khalid University Hospital (KKUH) and transferred into the violet vial containing EDTA as anticoagulant. This was centrifuged at 4000 rpm for 15 min, leaving the RBCs at the bottom, and WBCs, platelets (as grey buffy coat) and plasma (yellow–green liquid) on the top. Except the RBCs (about 2 ml), the supernatant was discarded. Samples of RBCs were prepared by saline water dilution, according to Parpart *et al.*[15]. This was used as the experimental sample.

One millilitre of RBC, taken in a closed, sterile tube, was irradiated for 24 h on both sides with the laser (He–Ne laser, $\lambda = 633$ nm, power 690 μW over a diameter of 3 mm), with frequent rotation of the tube for uniform exposure. This corresponds to an irradiance of 7.6 mW/cm^2 and a fluence of 59J. In a similar sterile tube 1 ml RBC was taken as control. The irradiated and control samples were kept at 4°C in a refrigerator. The OF of the above samples was monitored using absorption at 540 nm, according to a well-established protocol[15]. In short, the stronger the absorption at 540 nm, the greater the fragility of RBC. Such measurements were done every six days for the control and laser-irradiated samples. The instrument used was Perkin Elmer UV–Vis spectrometer which has a scan range 190–1100 nm.

A small quantity (100 μl) of the above blood samples was loaded in a RCL bridge for conductivity measurement, which has range from 10 Hz to 1000 MHz (PCA,

†This technique has won an US patent recently (US patent issued on 22 May 2014: Publication number: US 2014/0140889).

*For correspondence. (e-mail: dr.isam@gmail.com)

US make). In our experiment we have taken all the measurements for 10 laser-irradiated samples and 10 control samples. The whole experimental procedure was repeated thrice for consistency.

The OF of blood is a measure of the degradation of RBCs, using the absorption spectra at 540 nm (Figure 1). This is because the absorption peak at 540 nm is most sensitive to monitor the spectral features of haemoglobin, more precisely the porphyrin in the heme part of RBCs. The area of absorption at 540 nm is a measure of decomposition of RBCs and is presented as arbitrary units in Table 1. After 34 days of preservation, OF was 92% for control and 60% for the laser-irradiated sample. That is, there was about 55% retardation in the haemolysis of RBCs by laser biostimulation.

Figure 2 shows another way of representing the difference between the control and laser-irradiated samples. It shows haemolysis as a function of shelf life as monitored every six days. It can be seen from Figure 2 that half-life, defined as 50% haemolysis, is about 17 days for the control and 28 days for the laser-irradiated sample. That is, there is almost 65% enhancement in shelf life.

In order to understand the physical mechanism of decrease in haemolysis, which improves the shelf life, conductivity measurements were done. Figure 3 shows the conductivity as a function of log of frequency. Both samples show increase in conductivity; however for frequencies higher than 10 kHz, conductivity saturates for both samples. It can be seen that for any frequency, the laser-irradiation sample exhibits lower conductivity (or higher resistivity) and this difference is highest around 10 kHz.

It has been proved that measuring OF is a reliable way to monitor the viability and resilience of RBCs. This protocol has been used to diagnose blood diseases such as sickle cell anaemia and thalassaemia[16]. OF is directly

Table 1. Comparison of osmotic fragility of two red blood cells samples, control and irradiated with He–Ne laser, power 690 µW for 24 h

No. of days in preservation	Osmotic fragility (decay rate) of RBCs		
	Control sample	Laser-irradiated sample	Percentage improvement
10	36.23	10.84	69.70
16	52.02	24.61	52.15
22	69.35	35.11	49.13
28	79.82	50.43	37.00
34	91.66	59.38	34.00

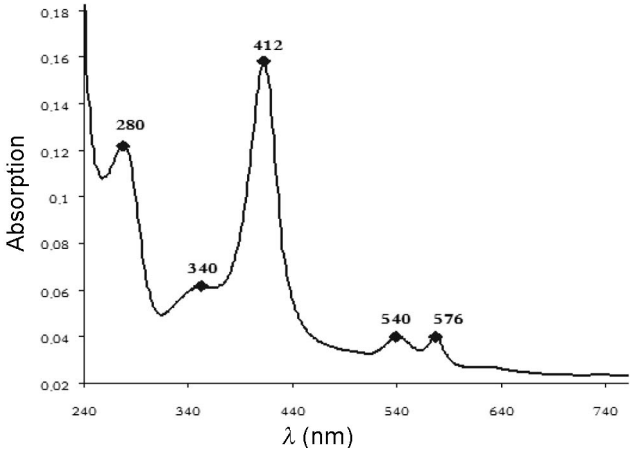

Figure 1. Absorption spectrum of red blood cells (diluted with normal saline) in the range 200–800 nm.

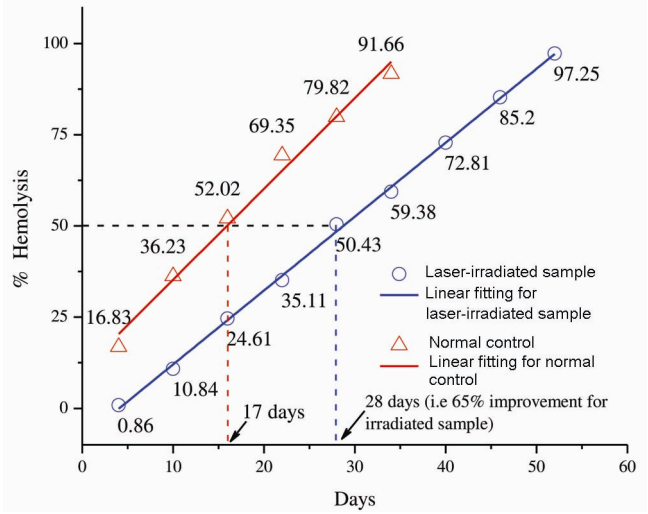

Figure 2. Relationship between fragility (decay rate) for two samples of RBC, control and irradiated, as a function of stored time in days. Note the half-time for haemolysis is 16 days for the control and 28 days for the irradiated sample.

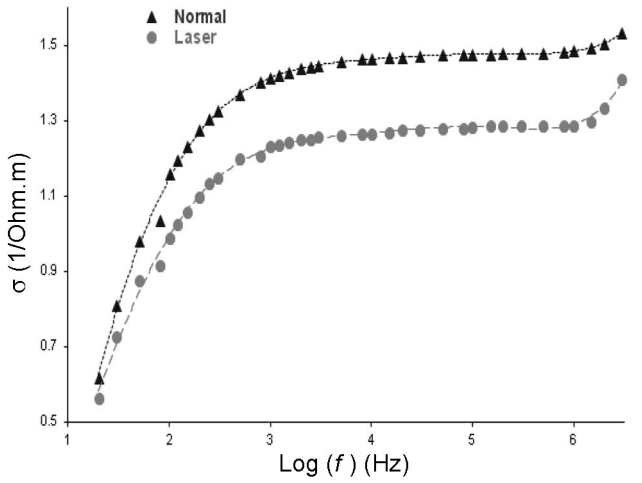

Figure 3. Plot of conductivity as a log of frequency. Both samples show increase in conductivity and saturate after 10 Hz. It can be seen that for any frequency the laser irradiation sample has lower conductivity (or higher resistivity) and this difference is highest at 10 kHz.

proportional to haemolysis and hence inversely proportional to cell viability. In the present study, RBC viability has been measured using the absorption at 540 nm. This is the characteristic band of haemoglobin, more particularly porphyrin in haeme. Our results had shown that laser-exposed samples have about 60% less absorption at 540 nm, i.e. 60% less haemolysis.

This could be due to increased resistivity as shown by the conductivity measurements. The cell membrane always contains static charges, because it is always in motion. This may polarize the inner constituents of cells, which may lead to haemolysis. It may be reasonable to assume that the fast electromagnetic field with a frequency of 10^{14} associated with LPLT, produces rapid (10^{14} Hz) alteration in the static charges built on membrane. Perhaps this could reduce the polarization-induced damage on the intracellular components. Such rapid change of charges could be equivalent thermal heating and cooling, which often lead to hardened surfaces in metallurgical annealing[17]. That is, LPLT is equivalent to rapid changes in the charge density (equivalent to electrical annealing), which increases the electrical resistivity of the membrane. Such a line of argument is strongly supported by our observation of 16% decrease in conductivity or increase in resistivity of the laser-exposed samples. Though the explanation offered here is hypothetical, the enhanced shelf life is factual.

In this study on shelf-life of whole blood is reported. It has been shown that low-power laser-irradiated RBCs show 60% enhancement in viability and lifetime. Such positive, beneficial changes could have been due to the 'electrical annealing' introduced by the low-power laser with electromagnetic waves of 10^{14} Hz. This technique may find clinical use as it is also economical.

1. Pavenski, K., Saidenberg, E., Lavoie, M., Tokessy, M. and Branch, D. R., Red blood cell storage lesions and related transfusion issues: a Canadian Blood Services research and development symposium. *Transfusion Med. Rev*., 2012, **26**, 68–84.
2. Sharifi, S., Dzik, W. H. and Sadrzadeh, S. M., Human plasma and tirilazad mesylate protect stored human erythrocytes against the oxidative damage of gamma-irradiation. *Transfusion Med. Rev.*, 2000, **10**, 125–130.
3. Ali, F. M., Mohamed, W. S. and Mohamed, M. R., Effect of 50 Hz, 0.2 mT magnetic fields on RBC properties and heart functions of albino rats. *Bioelectromagnetics*, 2003, **24**, 535–545.
4. Karu, T. I. and Anthony, T., Effects of low-power light on biological systems. In Proceedings of SPIE, Laser Use on Oncology, 2630, 1996.
5. Chichuk, T. V., Stranadko, E. P., Lubchenko, G., Podgornaya, V., Pozdnyakova, E. and Klebanov, G. I., Sensitized action of low-level laser radiation mechanism. In Proceedings of SPIE 4059, Laser Use on Oncology, 1999, vol. II, p. 112.
6. Brill, A. G., Brill, G. E., Shenkman, B., Tamarin, I., Dardik, R., Varon, D. and Savion, N., Low-power laser irradiation of blood inhibits platelet function: role of cyclic GMP. In International Society for Optics and Photonics, Biomedical Optics Conference, Europe, 1998, vol. 98, pp. 4–11.
7. Monika, O., Barbara, W., Maria, K. and Maria, B., The biostimulatory effect of red laser irradiation on big blood platelet function. *Cell Biol. Int.*, 1998, **22**, 245–248.
8. Khairullina, A. Y. and Oleinik, T. V., Biophysical information on the blood status upon irradiation by He–Ne laser. *J. Appl. Spectrosc*., 1996, **63**, 260–265.
9. Chichuk, T. V., Stranadko, E. P., Strashkevich, I. A. and Klebanov, G. I., Laser-induced priming of human blood leukocytes. In *Selected Papers on Laser Use in Oncology II*, International Society for Optics and Photonics, 1999, pp. 119–124.
10. Baranov, V. Y., Chekhov, D. I., Leonov, A. G., Leonov, P. G., Ryaboshapka, O. M., Semenov, S. Y. and Tatsis, G. P., Heat-induced changes in optical properties of human whole blood *in vitro*. In BiOS'99 International Biomedical Optics Symposium, International Society for Optics and Photonics, 1999, pp. 180–187.
11. Yova, D., Haritou, M. and Koutsouris, D., Antagonistic effects of epinephrine and helium–neon (He–Ne) laser irradiation on red blood cells' deformability. *Clin. Hemorheol*., 1994, **14**, 369–378.
12. Manteifel, V. and Karu, T., Activation of chromatin in T-lymphocytes under the He–Ne laser radiation. *Proc. SPIE*, 1999, **74**(1), 12–16.
13. Mi, X. Q., Chen, J. Y., Cen, Y., Liang, Z. J. and Zhou, L. W., A comparative study of 632.8 and 532 nm laser irradiation on some rheological factors in human blood *in vitro*. *J. Photochem. Photobiol.,* 2004, **74**, 7–12.
14. Mascheroni, M., Extending the shelf life of donated blood. Innovation, 2008, **6**(3); http://www.innovation-america.org/extending-shelf-life-donated-blood (accessed on 6 September 2015).
15. Parpart, A. K., Lorenz, P.B., Parpart, E. R., Greegg, J. R. and Chase, A. M., The osmotic resistance (fragility) of human red cells. *J. Clin. Invest*., 1947, **26**, 636–640.
16. Ibegbulem, C. O., Eyong, E. U. and Essien, E. U., Polymerization inhibition activity of *Raphia hookeri* palm sap and its effect on osmotic fragility of sickle cell red blood cells. *J. Med. Plants Res*., 2011, **5**, 4212–4217.
17. Negres, R. A., DeMange, P. and Demos, S. G., Investigation of laser annealing parameters for optimal laser-damage performance in deuterated potassium dihydrogen phosphate. *Opt. Lett*., 2005, **30**, 2766–2768.

ACKNOWLEDGEMENT. We thank the Deanship of Scientific Research at King Saud University for financial support through a research group project (project number: RGP-VPP-223).

Received 15 January 2015; revised accepted 31 May 2015

doi: 10.18520/v109/i6/1151-1153