Rapid publication

Dielectrophoretic separation and enrichment of CD34+ cell subpopulation from bone marrow and peripheral blood stem cells

M. S. Talary[1] K. I. Mills[2] T. Hoy[2] A. K. Burnett[2] R. Pethig[1]

[1] Institute of Molecular and Biomolecular Electronics, University of Wales, Dean Street, Bangor, Gwynedd LL57 1UT, UK
[2] University of Wales College of Medicine, Department of Haematology, Health Park, Cardiff CF4 4XN, UK

Abstract—*Dielectrophoresis has been used to enrich selected cell subpopulations in a mixed cell population by exploiting differential dielectric properties. Six-fold enrichment of stem cells expressing the CD34+ antigen has been achieved for bone marrow samples and peripheral blood, without the requirement for initial chemical treatment associated with immunoadsorption techniques.*

Keywords—*Cell Separation, CD34+, Dielectrophoresis, Enrichment, Transplantation*

Med. & Biol. Eng. & Comput., 1995, 33, 235–237

1 Introduction

THERE IS currently considerable biomedical and clinical interest in isolating the cell subpopulation of bone marrow (BM) and peripheral blood stem cell collections (PBSC), which contains the haematopoietic stem cells required for bone marrow reconstitution. The CD34+ antigen, a marker that has been used to identify haematopoietic precursor cells, is expressed most strongly on these primitive cells and is progressively lost as the cells differentiate. Studies of cell ancestry from this common precursor are hampered by their low frequency, with the CD34+ antigen expressed on 1%–4% of BM and PBSC that includes the haematopoietic colony forming cells (BENDER *et al.*, 1991), and by the lack of a means of direct identification and enumeration.

We show that enrichment of CD34+ cells that contain the stem cell subpopulations in bone marrow and peripheral blood stem cell samples can be achieved using dielectrophoresis, which is the force exerted on particles subjected to non-uniform AC electrical fields (POHL, 1978). The significance of this work presented here is that, although dielectrophoresis has recently been used for the separation of prepared mixtures of micro-organisms (MARKX *et al.*, 1994 a; b) and mammalian cells (GASCOYNE *et al.*, 1992; 1993; BECKER *et al.*, 1994a; b), we show for the first time that dielectrophoretic separation can be achieved for natural biological samples.

2 Materials and methods

2.1 *Separation chamber construction*

The separation chamber incorporates gold-plated interdigitated castellated electrodes, fabricated using standard photolithography by vacuum evaporation of a 100 nm layer of gold onto a 5 nm thick seed layer of chromium on glass microscope slides (PRICE *et al.*, 1988). The electrodes are of total surface area 12 cm^2, with a castellated geometry of characteristic feature size 80 μm. The method of chamber construction is similar to that used in the dielectrophoresis spectrometer described previously (TALARY and PETHIG, 1994).

2.2 *Cell sample preparation*

Cell samples were prepared for separation by taking 0·5 ml and 0·25 ml of peripheral blood from stem cell harvests and bone marrow, respectively, and making the solution up to 20 ml with PBS (Phosphate Buffered Saline). A lymphocyte density gradient separation technique* is used to remove the red blood cells (RBC). The remaining leucocytes are washed and resuspended three times in PBS by centrifuging at 400 g for 5 min. For the final wash they are resuspended in freshly prepared 320 mM sucrose solution containing 3 mg ml^{-1} glucose, after first carefully washing away any traces of the PBS.

First received 29 November 1994.

* Histopaque 1077, Sigma Chemicals

2.3 *Separation method*

The first stage of the separation process consists of applying a sinusoidal voltage to the electrodes, using a frequency at which all cells in the mixed sample were collected in 'pearl chains' at the electrode tips under the influence of positive dielectrophoresis. The pump to the sample chamber is turned on, and the lymphocyte sample flows into the chamber with the electrodes energised by a 6 V pk-pk sinusoidal waveform at a frequency of 500 kHz. Cells are collected within the collection chamber for 10 min with this field constantly applied. Peristaltic pumps are used alternately to pump cells through the separation chamber (as shown in Fig. 1).

The pump to the sample chamber is then turned off, and cell-free suspending fluid is pumped into the separation chamber to flush out any cellular debris and excess cells that are not captured by the electrodes. This also enabled the required suspending medium conductivity to be altered and maintained within the separation chamber. Cell fractions can then be collected from the output of the separation chamber as the frequency of the applied field is varied. The applied field frequency is then reduced to a region where there is a reduction in the positive dielectrophoretic force and the cells begin to undergo negative dielectrophoresis. Cell fractions are collected at 10 min intervals after the applied field frequency is reduced and are labelled with fluorescent cell markers for the CD34+ antigen. Subsequent FACS analysis is used to quantify the percentage cell population of CD34+ within each collected fraction.

2.4 *Cytometry*

1 ml of cells in the suspending medium is washed with 5 ml of PBS containing 1% bovine serum albumen (BSA) and centrifuged at 250 g for 10 min. The supernatant is decanted, and the cells are resuspended in 100 μl and left for 12 h at 4°C in the dark. Samples are divided and incubated for 30 min at 4°C with antibodies (HPCA-2 and isotype control) directly conjugated with phycoerythrin†. Cells are then washed twice with 2 ml of PBS/BSA as above and finally resuspended in 300 μl for analysis on a FACScan§ flow cytometer. Data are collected on 5000 events for each sample.

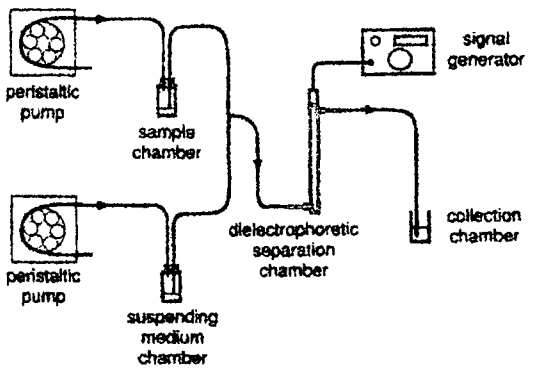

Fig. 1 *Apparatus for dielectrophoretic separation and enrichment of bone marrow*

†§ Becton Dickinson, San Jose, California

3 Results and discussion

The relative concentration of the CD34+ stem cells in the BM and PBSC samples is initially determined by allowing the cell suspension to flow through the dielectrophoretic chamber with no applied voltage to the microelectrodes. This is followed by standard FACS analysis where the cells are labelled with a fluorescent antibody against the CD34+ antigen present on the surface of the progenitor stem cells. Cell separation and enrichment of the CD34+ subpopulation within BM and PBSC is achieved in two stages. Initial collection is achieved by energising the electrodes with a sinusoidal voltage (6 V pk-pk, 500 kHz) where all the cells experienced a high positive dielectrophoretic force, thus trapping them at the electrode edges. Subsequently, a cell-free solution (320 mM sucrose and 3 mg ml^{-1} glucose) of electrical conductivity 10 $\mu S\ cm^{-1}$ is pumped through the chamber, and the AC voltage energising the electrodes was reduced from 500 kHz down to 1 kHz in stages of 10 min duration. As the applied field frequency is reduced, the magnitude of the dielectrophoretic force is reduced at a rate which differs according to cell type (WANG *et al.*, 1994), and so differential retention of the cells occurs within the separation chamber.

A dot plot of the forward laser scatter versus the side scatter reveals a recognised pattern where lymphocytes can be clearly identified and gated. By examining the fluorescence of cells within this gated region containing the lymphocytes, the relative distribution of the high fluorescent CD34+ species is then determined. Fig. 2 shows the relative fluorescent intensity of cells within each fraction collected at the different set frequencies. At an applied frequency of 500 kHz, the histogram reveals that highly fluorescent CD34+ cells are absent, indicating that they were dielectrophoretically trapped within the separation chamber. As the frequency of the applied field is further reduced, the CD34+ cells are seen to be eluted below 50 kHz, with the maximum elution occurring at 5 kHz.

The number of cells that fall within the high fluorescent intensity region in the gated histograms can be determined by setting markers in the region where events related to the CD34+ cells occur, and quantifying these regions as a percentage of the total number of recorded events (5000). Fig. 3 shows the number of cells present in the marked region as a percentage of the total fraction of gated events. The initial sample shows that the CD34+ subpopulation accounts for 0·84% of the fraction, which is within the expected range for peripheral blood samples. Subsequent fractions at 500 kHz and 100 kHz show the absence of the CD34+ antigen, indicating that dielectrophoretic collection enables this subpopulation to be selectively retained within the separation chamber. The cell fraction collected at 5 kHz displays the presence of a 4·97%

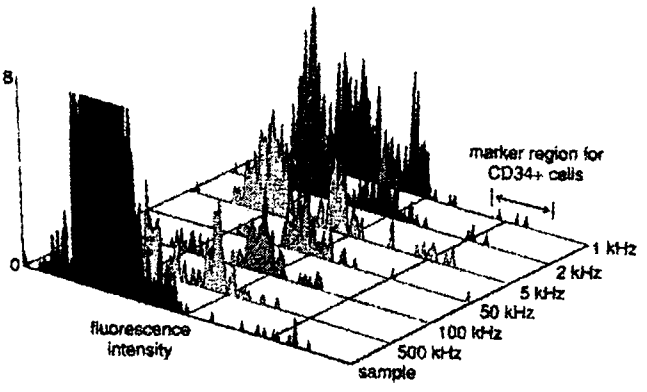

Fig. 2 *Histograms of lymphocyte gated region showing number of events and relative fluorescent intensity in fractions collected at various frequencies related to presence of CD34+ antigen*

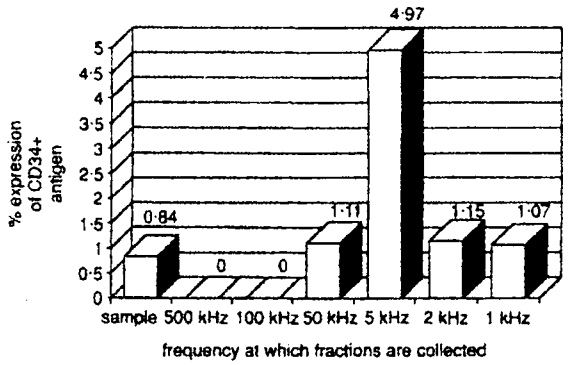

Fig. 3 *Dielectrophoretic separation efficiency*

population of CD34+ cells, representing a 5·9-fold enrichment of stem cells. Subsequent reprocessing of the sample or the use of multiple cascaded chambers would improve this enrichment. Our current efforts are also directed to achieving improved separation efficiency by investigating the influence of flow rate, applied voltage and conductivity of the suspending medium.

Acknowledgments—This work was supported by the HEFCW Collaborative Research Initiative, the National Foundation for Cancer Research and by a donation from the Laura Ashley Social Club, Caernarfon.

The authors would like to thank R. Bailey-Wood for providing the BM and PBCS samples; J. Tame for fabricating the microelectrodes; and Dr. J. P. H. Burt and Dr. G. H. Markx for valuable discussions.

References

BECKER, F. F., WANG, X.-B., HUANG, Y., PETHIG, R., VYKOUKAL, J., and GASCOYNE, P. R. C. (1994a): 'Separation of human breast cancer cells from blood by differential dielectric affinity'. *Proc. Nat. Acad. Sci. USA*

BECKER, F. F., WANG, X.-B., HUANG, Y., PETHIG, R., VYKOUKAL, J., and GASCOYNE, P. R. C. (1994b): 'The removal of human leukaemia cells from blood using interdigitated microelectrodes'. *J. Phys. D: Appl. Phys.*

BENDEER, J. G., UNVERZAGT, K. L., WALKER, D. E., LEE, W., VAN EPPS, D. E. SMITH, D. H., STEWART, C. C., and BIK, TO, L. (1991): 'Identification and comparison of CD34 positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry.' *Blood*, **77**, pp. 2591–2596

GASCOYNE, P. R. C., HUANG, Y., PETHIG, R., VYKOUKAL, J., and BECKER, F. F. (1992): 'Dielectrophoretic separation of mammalian cells studied by computerized image analysis,' *Meas. Sci. Technol.*, **3**, pp. 439–445

GASCOYNE, P. R. C., PETHIG, R., BURT, J. P. H., and BECKER, F. F. (1993): 'Membrane changes accompanying the induced differentiation of Friend murine erythroleukaemia cells studied by dielectrophoresis.' *Biochim. Biophys. Acta*, **1149**, pp. 119–126

GASCOYNE, P. R. C., NOSHARI, J., BECKER, F. F., and PETHIG, R. (1994): 'Use of Dielectrophoretic Collection spectra for characterising differences between normal and cancerous cells,' *IEEE Trans.*, **IA-30**, pp. 829–834

MARKX, G. H., HUANG, Y. ZHOU, X.-F., and PETHIG, R. (1994a): 'Dielectrophoretic characterization and separation of micro-organisms,' *Microbiol.*, **140**, pp. 585–591

MARKX, G. H., TALARY, M. S., and PETHIG, R. (1994b): 'Separation of viable and non-viable yeast using dielectrophoresis,' *J. Biotechnol.*, **32**, pp. 29–37

POHL, H. A. (1978): 'Dielectrophoresis' (Cambridge University Press, Cambridge, UK)

PRICE, J. A. R., BURT, J. P. H., and PETHIG, R. (1988): 'Applications of a new optical technique for measuring the dielectrophoretic behaviour of micro-organisms,' *Biochim. Biophys. Acta*, **964**, pp. 221–230

TALARY, M. S., and PETHIG, R. (1994): 'Optical technique for measuring the positive and negative dielectrophoretic behavior of cells and colloidal suspensions,' *IEE Proc. Sci. Meas. Technol.*, **141**, pp. 395–399

WANG, X.-B., HUANG, Y. HÖLZEL, R., BURT, J. P. H., and PETHIG, R. (1993): 'Theoretical and experimental investigations of the dielectric, dielectrophoretic and electrorotational behaviour of colloidal particles,' *J. Phys. D. Appl. Phys.*, **26**, pp. 312–322

WANG, X.-B., HUANG, Y., GASCOYNE, P. R. C., BECKER, F. F., HÖLZEL, R., and PETHIG, R. (1994): 'Changes in Friend murine erythroleukaemia cell membranes during induced differentiation determined by electrorotation,' *Biochim. Biophys. Acta*, **1193**, pp. 330–344