Isolation of Cord Blood-Derived Hematopoietic Stem Cells Using Macroporous Affinity Cryogel Matrix

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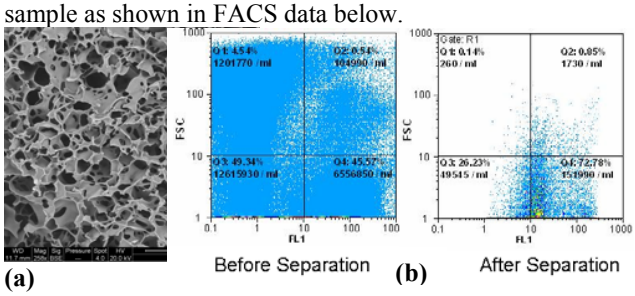
Statement of Purpose: There is a growing interest in cord blood as a source of primitive stem cells with the capacity for multilineage differentiation. Pure cell fractions are needed for the characterization and *in vitro* expansion of stem cells as well as for their use in preclinical research. However, enrichment of stem cells is challenging due to the lack of gentle protocols for the isolation of highly pure stem cell fractions. Protocols developed for the enrichment of peripheral blood-derived stem cells have been found to be suboptimal for cord blood. New monolithic supermacroporous matrices with pore size upto 200µm have been developed and utilized for various applications. These matrices are produced by crosslinking polymerization of the hydrophilic/ hydrophobic monomers/polymers in water based solvent at subzero temperatures. Our group has successfully demonstrated the application of these matrices for the separation of different cell types by utilizing the affinity of the cell surface receptors for different ligands [1]. An interesting characteristic of these macroporous cryogels have been its sponginess [2], which allows the affinity bound cells to be released from the matrix by gentle mechanical squeezing of the gels [3]. The affinity ligand used to capture specific cell types are usually monoclonal antibodies. The present work includes designing of macroporous polymeric cryogels as cell chromatography matrix for stem cell separation using anti-$CD34^+$ monoclonal antibody.

Methods: The epoxy-activated poly(acrylamide) (PAAm) and poly(N-isopropyl acryl amide) (PNiPAAm) cryogel were synthesized at -12 °C and dried in vacuum for further experiments. The physical characterizations were done including porosity by mercury porosimetry and scanning electron microscopy(SEM). The flow rate, mechanical characterization and swelling studies were also tested. The protein A was then coupled to epoxy-activated cryogel matrix for immobilizing anti-$CD34^+$ through its Fc portion. The umbilical cord blood received from city hospital under sterilized condition for further experiments. The collected blood was then labelled with anti-$CD34^+$ and captured on affinity cryogel column. The cells were then eluted by two different methods (i) released by mechanical squeezing and (ii) by using immunoglobulin (IgG) displacement. The eluted CD34+ cells were observed by FACS analysis. The cell viability was measured by trypan blue staining and FACS. The isolated stem cells were cultured in low glucose DMEM cell culture media for 1-2 weeks and the proliferation was checked by MTT assay. The oct3/4 gene analysis was done to prove the proliferation of isolated hematopoietic stem cells.

Results: The PAAm and PNiPAAm cryogel shows large pore size upto 200µm. The SEM analysis demonstrates the interconnected porosity which is further confirmed by mercury porosimeter analysis and swelling studies. The pore distribution data from mercury porosimeter revealed large number of pores lying in the range of 30-70µm. The swelling ratio ranges from 14-20 depending upon the polymer concentration. The swelling kinetics demonstrates the interconnected porosity due to its quick swelling in water which was within 1min. The cryogel had high flow rate of upto 5ml/min. Mechanically PNiPAAm cryogel was spongier than PAAm cryogel with Young's modulus 33–65 kPa and 42–86 kPa respectively. The anti-CD34+ labeled umbilical cord blood when passed through the protein A coupled PAAm and PNiPAAm cryogel column caused the attachment of stem cells specifically on matrix and the analysis of eluted samples from the matrix showed the enrichment of CD34+ and removal of other cells present in loaded blood sample as shown in FACS data below.



(a) **(b)**

Figure 1. SEM images. (a) PAAm cryogel and (b) FACS analysis of isolation of CD34+ cells before and after separation.

The high cell viability and yield demonstrate the successful cell chromatography procedure for stem cell separation. The isolated cells were then checked for its viability by trypan blue staining and it was found that more than 80% cells were viable. The proliferation of stem cells was measured by MTT assay and there was increase in cell number checked over a period of 15 days. In final experiments the grown cells were then subject to oct3/4 gene analysis to prove the proliferation capacity of stem cells and to provide as marker for hematopoietic stem cells with self renewal capacity.

Conclusions: The supermacroporous cryogels with specific ligand for cell chromatography demonstrate the separation of stem cells umbilical cord blood. This may provide useful approach in preparative cell separation for its application in stem cell based cell therapy, cell-based immune therapy and for other biomedical application. These results further confirmed that developed cryogel matrices are suitable for cell separation application. These cryogel chromatography matrices can be used for whole blood processing.

References: (1) Kumar A. J Immunol Methods 2003; 283:185.
(2) Srivastava A. Materials Science and Engineering A 2007;464:93.
(3) Dainiak MB, Kumar, A Proc Natl Acad Sci USA 2006;103:849.