THE SEPARATION OF RED BLOOD CELLS BASED SOLELY ON INTRINSIC MAGNETIZATION: CLINICAL AND COMMERCIAL IMPLICATIONS

Jeffrey J. Chalmers, The Ohio State University
Chalmers.1@osu.edu
Jenifer Gomez Pastora, The Ohio State University
James Kim, The Ohio State University
Mitchel Weigand, The Ohio State University

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A rough estimate puts the cell isolation market at approximately $ 6 billion a year worldwide. One of the key commercial technologies uses antibodies conjugated to magnetic micro and nanoparticles (i.e Dynal beads or Miltenyi MACS systems). While clearly effective, whenever antibodies are used, whether conjugated to magnetic particles, or fluorescent molecules (such as used in FACS systems), there is always the issue of the sensitivity and specificity of the antibody for the targeted cell(s). This “issue”, amongst others, is the motivator for “label free” identification and separation technology.

Removal of human red blood cells, hRBCs, from a blood or bone marrow sample for diagnostic, or therapeutic applications is a fundamental laboratory practice/procedure. While difficult to obtain precise numbers, it has been suggested that greater than a billion blood draws are conducted in the US each year. While for a majority of these blood draws an evaluation of the RBCs is an important part, there is still a very large number of tests that focus on the remaining blood components after the RBCs have been removed. While not nearly as common as a blood draw, more than 18,000 bone marrow or umbilical cord blood transplants were performed in the US in 2013. In the case of bone marrow transplants, the RBCs need to be removed prior to transfusion or cryopreservation, regardless of whether the donor and patient’s tissues match.

Viewed from a mechanistic perspective, there are three primary methodologies to remove human RBCs, hRBCs, from a blood draw: 1) RBC lysis, 2) immunological based separation in which a RBC is bound with an affinity ligand which facilitates RBC removal, or 3) separation of the RBC from the nucleated cells based on density differences. The two most commonly used methods are the density difference methods with or without hydrophilic polysaccharide addition (e.g Ficoll density gradient based centrifugation, DGC,). When blood samples are only used for further analysis, the condition and the content of the sample after the RBC removal is only important with respect to how it affects the subsequent analysis; however, when the RBC depleted sample is destined for transfusion into a patient, significantly higher standards are required.

We previously compared RBC removal using the Ficoll-based DGC to lysis protocols. Using either method would remove more than 99% of RBCs; however the average recovery of the spiked cancer cells was 73 and 89% for the Ficoll and RBC lysis, respectively. Poor recovery of targeted cells, such as hematopoietic stem cells, in the initial RBC depletion step is a problem in the bone marrow transplant/regenerative medicine community. In fact, several reports indicate that the recovery of nucleated cells from bone marrow, BMNCs, using Ficoll-based DGC, can be as low as 15-30%. Complementary to these reports, two recent papers suggest that cells with high regenerative potential, such as very small embryonic-like stem cells, VSELs and mesenchymal stromal cells are depleted with DGC. Finally, there are suggestions that Ficoll DGC can impair receptor function of the recovered cells.

It is well established that deoxygenated RBCs are weakly paramagnetic; initially reported by Linus Pauling and coworkers in 1936. Melville and co-workers demonstrated in the mid 1970’s that RBCs can be captured using a ferromagnetic wire mesh when the cells are reduced (chemical turned into a state equivalent to the deoxy-state). More recently, we have demonstrated that RBCs can be captured in HGMS systems (i.e. Miltenyi Biotec MACS columns), magnetically deposited on slides, deposited on the wall of a channel, and continuous removed using a flow through separation system. While these studies demonstrate theoretically, and experimentally, that it is possible to separate RBCs based on intrinsic magnetization, the throughputs in these studies are orders of magnitude lower than needed to practically remove RBCs from a typical blood draw.
In this presentation we will present our latest systems which we suggest can increase the throughputs by orders of magnitude which presents the potential for magnetic separation of RBCs to become a practical alternative to the currently used approaches.