

2ND INTERNATIONAL CONFERENCE ON CELL SCIENCE AND REGENERATIVE MEDICINE



**Zeeshan Afzal^{1*}, Kourosh Saeb-Parsy¹,
Emmanuel Huguet¹**

1 Cambridge University, Department of Surgery, Addenbrookes Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge NIHR Biomedical Research Centre, Hills Road, Cambridge CB20QQ, UK

Isolation of CD133 positive cells from rat bone marrow by immune-magnetic beads and Fluorescence-activated cell sorting

Abstract: In the rat model, following liver injury, a significant proportion of liver sinusoidal endothelial cells (LSECs) proliferating in regenerating liver are derived from CD133+ bone marrow (BM) progenitors, which proliferate in the BM, migrate into the blood stream and engraft into the liver, where they differentiate into mature LSECs, and express high levels of Hepatocyte Growth Factor, thus promoting hepatocyte proliferation. The study aim was to isolate CD133+ cells from rat BM using (1) direct magnetic bead separation with human CD133 antibody conjugated magnetic beads, (2) indirect magnetic bead separation using FITC-labelled rat specific CD133 antibody and anti-FITC conjugated magnetic beads and (3) Fluorescence-activated cell (FACS) sorting with FITC labelled rat specific CD133, and compare efficacy and practicality of these techniques.

Rat BM cells were labelled with FITC conjugated rat specific anti CD133 antibody and further processed for either method 1, 2 or 3. Direct magnetic bead separation produced poor enrichment of rat CD133+ cells. This is likely due to poor affinity of human magnetic beads towards rat CD133 molecule resulting in loss of cells. In contrast, indirect magnetic bead separation with rat specific FITC-labelled CD133 antibody and anti FITC magnetic beads, or FACS sorting both resulted in enhanced rat CD133+ cell enrichment. Although similar in amino acid sequence, the differences between human and rat CD133 molecules are may be sufficient to result in poor binding of the human CD133 antibody to the rat homolog molecule. Consequently, whilst effective in enriching human CD133+ cells, magnetic beads conjugated to human CD133 do not produce effective enrichment of rat CD133+ cells. In contrast, rat specific CD133 antibody-based methods result in effective CD133+ cell enrichment from rat BM.

Keywords: Regenerative; Bioengineering; Liver; Decellularisation; CD133, magnetic beads, Fluorescence-activated cell sorting

Biography: Zeeshan Afzal is a specialty registrar in the run-through General Surgery training program at Addenbrooke's Hospital, University of Cambridge, and a member of the Royal College of Surgeons of England. He completed his medical degree at the University of Leicester, followed by further clinical and surgical training in Cambridge. He is currently working in the Department of Transplant Surgery at Addenbrooke's Hospital and is pursuing a PhD at the University of Cambridge, focusing on bioengineering liver tissue using bone marrow progenitor cells. Zeeshan aspires to become a consultant in Hepato-Pancreato-Biliary and Transplant Surgery, with a special interest in regenerative medicine.