DIFFERENCES IN MORPHOLOGY, PROLIFERATION AND IMMUNE PROFILE AMONG SINGLE-CELL CLONED STEM CELLS FROM THE SAME MESENCHYMAL STEM CELL ORIGIN

Y. Cheng, S. Samizadeh, M. Coathup, G. Blunn

John Scales Centre for Biomedical Engineering
Institute of Orthopaedics and Musculoskeletal Science
Division of Surgery and Interventional Science
Royal National Orthopaedic Hospital
University College London
Brockley Hill, Stanmore, Middlesex HA7 4LP

Introduction: Mesenchymal stem cells (MSCs) are usually believed to be immune-privileged. However, immunogenic MSCs have also been reported. We hypothesize that there are differences between MSC clones from the same individual in terms of their morphology, proliferation, differentiation and immunogenicity. Our goal is to discover a source of immune-privileged stem cells for universal allogenic MSCs transplantation.

Materials and Methods: Serial dilutions of bone-marrow derived (BMMSCs) and adipose derived mesenchymal stem cells (ADMSCs) from same animal were carried out to isolate single-cell clones. From a single animal we obtained 3 clones from BMMSCs and 3 from ADMSCs. The proliferation rate of each clonal culture and mixed clonal culture were measured. The tri-differentiation potential of the clonal cultures were compared and a comparison was also made with the original isolates from bone marrow and fat. The immune-privileged properties were measured by flow cytometry and immuno-staining for the major histocompatibility complex (MHC) antigens. Mixed leucocyte reactions (MLR) were performed to investigate immunogenicity.

Results: Tri-differentiation was confirmed in all isolates. All clonal cultures revealed individual morphology and significantly different proliferation rates, compared with each other and mixed cultures (figure1, table 1). All clonal cultures showed different surface markers, inclusive of MHC antigens. One clone from ADMSCs showed lack of MHC antigens. Our MLR and MHC staining disclosed a variety of immune responses and markers.

Discussion and Conclusion: All clones tri-differentiated which indicated a degree of 'stemness'. MSCs are generally believed not to express MHC II, resulting in immune-privileged. Our results confirmed our hypothesis because clonal cultures isolated from different origins of same animal show differences in morphology, proliferation rate, and surface marker presentation. Individual immune differences highlighted through single-cell clonal cultures may be crucial to find universal, immune-privileged MSCs as universal allogeneic donor.

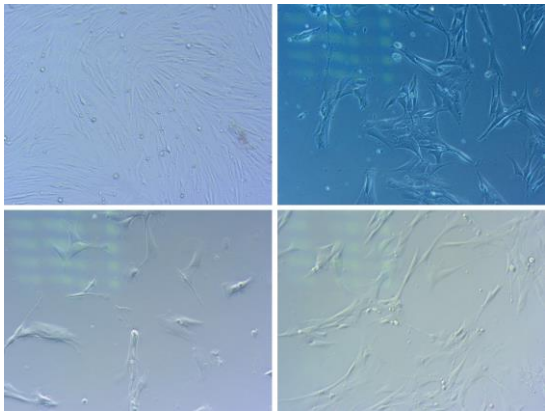

Figure 1: Different morphology of single-cell cloned MSCs.
Left & right upper: from adipose derived mesenchymal stem cells
Left & right lower: from bone marrow MSCs.

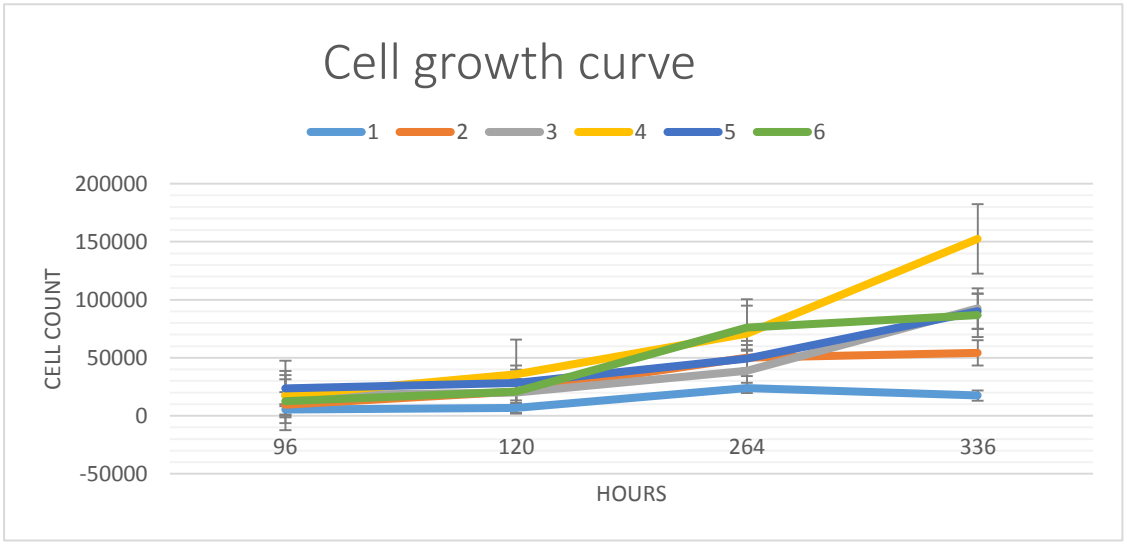

Table 1: Single-cell clones MSCs growth curve. Group 1,2,3 are from bone marrow MSCs. Group 4,5,6 are from adipose derived MSCs. Initial seeding density is 10000 cells per well in 24-well plate.