Regenerative Medicine category

**ASSESSING THE CHARACTERISTICS OF HUMAN MESENCHYMAL STROMAL CELLS DERIVED FROM BONE MARROW AND UMBILICAL CORD FOLLOWING EXPANSION IN THE QUANTUM**® **HOLLOW-FIBRE BIOREACTOR SYSTEM.**

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**Abstract**

**Purpose**

The Quantum® bioreactor (Terumo BCT) is an automated hollow fibre system, which has an internal surface area of 2.1m2 (equivalent to 120 T-175 tissue culture flasks), allowing large scale expansion of cells. The purpose of this study was to assess the characteristics of mesenchymal stromal cells (MSCs) derived from human bone marrow (BM) and umbilical cord (UC) after expansion in the system compared to the standard tissue culture plastic technique (TCP), with the aim of using these cells in cell therapies for cartilage repair.

**Methods**

Quantum® fibers were coated with human cryoprecipitate (NHS Blood & Transplant) prior to cell seeding.. The bioreactor (Fig. 1) was loaded with 20ml of BM and cells were harvested and characterised at the end of the first passage. 5-10 M of these cells were then re-seeded into the Quantum® for a second expansion. The Quantum® is best suited to the loading of high cell numbers; hence a ‘hybrid’ process was used for UC-MSCs, whereby UC-MSCs were isolated from UC matrix and expanded first on tissue culture plastic for ~14 days; after this time 5 M cells were loaded into the bioreactor. A total of four BM donors and four UC donors were used individually in the system.

Flow cytometry was used to assess the MSC immunoprofileof each Quantum® cell product according to the International Society for Cell Therapy (ISCT) definition of MSCs, as well as a panel of chondrogenic markers (CD44, CD166, CD49c, CD39, CD151, CD271, FGFR3 and ROR2). A panel of markers indicative of immunogenicity (CD40, CD80, CD80, and HLA-DR) and immunomodulation (CD317 and CD106). CD317 and CD106 were also assessed before and after inflammatory stimulation with IFN-ϒ (25ng/ml, 24 h). Further, each Quantum® product was assessed for its chondrogenic capacity using a standard pellet culture differentiation protocol. In addition, a live cell imaging platform (Cell IQ, CM Technologies) was used to assess each BM cell product at both passages and a commercial ELISA was used to quantitate IL-8 in the BM-cell secretome (conditioned medium) as a possible indicator of the level of inflammatory cells present at P1 and P2.

**Results**

BM-MSC harvests after the first passage (mean 14.2 ± 1.5 days in culture) were: 10M, 14M 19M and 47M. Flow cytometry, secretome analysis and live cell imaging possibly indicated a 15-30% macrophage contamination (Fig.2). Secretome analysis of BM-MSCs during the primary and secondary expansion in the Quantum® system showed that for each of the donors tested IL-8 was detected only in the conditioned medium from the primary expansion and was undetectable during the second expansion.

BM-MSC harvests after the second passage (mean 13 ± 1.4 days in culture) were: 25M, 105M, 176M and 218M and UC-MSC harvests after the second passage (mean 7.7 ± 2.2 days in culture) were: 90M, 188M, 194M and 200M. BM-MSCs and UC-MSCs had mean doubling times of 2.1 ± 0.67 and 1.06 ± 2.2 days, respectively after the second passage.

Flow cytometry (Fig.3) and live cell imaging indicated that Quantum® expanded BM-MSCs and UC-MSCs at second passage adhered to the ISCT criteria for MSCs and had comparable chondrogenic potency and immunomodulatory immunoprofiles before and after pro-inflammatory stimulation. Cell characterisation of Quantum® and TCP expanded BM-MSCs and UC-MSCs were similar, with no obvious differences.

Conclusions

These preliminary results suggest that the Quantum® system can successfully be used to expand large numbers of MSCs from bone marrow and umbilical cord tissues. Further *in vitro* and *in vivo* work will establish the multi-lineage capacity of these cells and the effect of inflammatory cells or macrophages present in the BM-MSC population at P1 on the potential of these cells for use in orthopaedic cell-based therapies for cartilage repair.

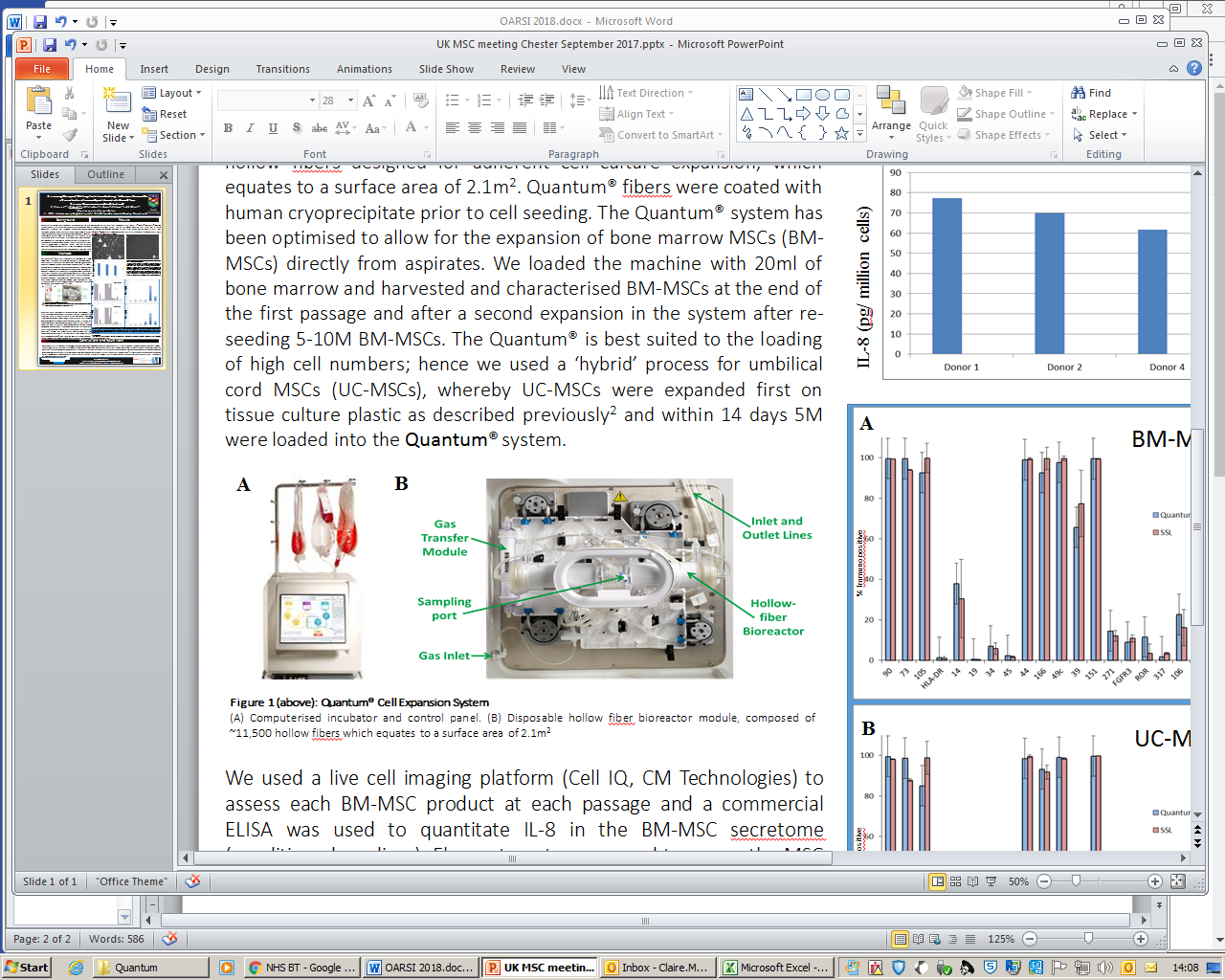


Figure 1. The Quantum® cell expansion system. A. Computerised incubator and control panel. B. Disposable hollow fibre bioreactor module composed of ~11,500 fibres.

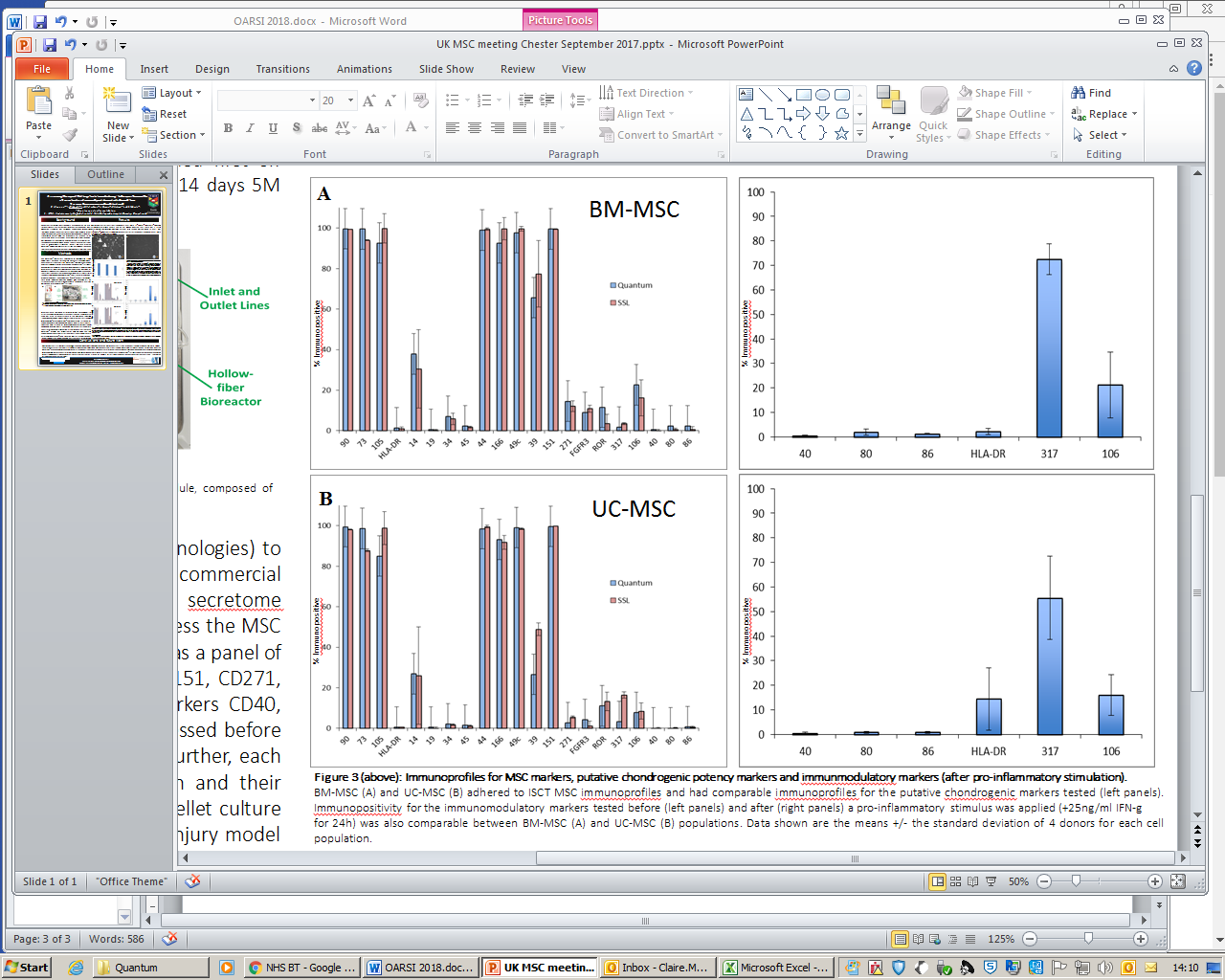


Figure 2. A. The appearance of a macrophage like ‘fried egg’ morphology at P1 in BM-MSC cultures from the Quantum® (white arrows). B. BM-MSCs after P2 in the Quantum® showing a typical homologous MSC morphology. C. Conditioned medium was collected from secretome analysis during the primary and secondary expansion in the Quantum® system for three of the BM-MSC donors included in this study (the fourth donor had a lower cell count). For each of the donors tested IL-8 was only detected in the conditioned medium collected during the primary expansion and was undetectable during the second expansion phase.

TCP

Quantum

Quantum

TCP