Characterising Cellular Components of Bone Marrow Aspirate Concentrate – A One-Step Cell Therapy for Ankle Cartilage Defects

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Objectives

Bone marrow aspirate concentrate (BMAC), together with fibrin glue (Tisseel, Baxter, UK) and Hyaluronic acid (HA) were used as a one-step cell therapy treating patients with ankle cartilage defects in our hospital. This therapy was proven to be safe, with patients demonstrating a significant improvement 12 months post-treatment. Enriched mesenchymal stem cells (MSCs) in BMAC are suggested inducers of cartilage regeneration, however, currently there is no point-of-care assessment for BMAC quality; especially regarding the proportion of MSCs within. This study aims to characterise the cellular component of CCR-generated BMAC using a point-of-care device, and to investigate if the total nucleated cell (TNC) count and patient age are predictive of MSC concentration.

Methods

During surgery, 35ml of bone marrow aspirate (BMA) was collected from each patients’ iliac crest under anaesthesia, and BMAC was obtained via a commercial kit (Cartilage Regeneration kit, CCR, Innotec®, UK). BMAC was then mixed with thrombin (B+T) for injection with HA and fibrinogen.

In our study, donor-matched BMA, BMAC and B+T were obtained from consented patients (n=12, age 41 ± 16years) undergoing surgery with BMAC therapy. TNC, red blood cell (RBC) and platelet (PLT) counts were measured via a haematology analyser (ABX Micros ES 60, Horiba, UK), and the proportion of MSCs in BMA, BMAC and B+T were assessed via colony forming unit-fibroblast (CFU-F) assays. Significant differences data in matched donors were tested using Friedman test. All data were shown as mean ± SD.

Results

Mean TNC counts in BMA and BMAC were not significantly different (14.0 ± 4.4 million/ml and 19.4 ± 32.9 million/ml, respectively, *P*>0.9999). However, TNC counts were significantly lower in B+T compared to BMAC (9.7 ± 24.5 million/ml and 19.4 ± 32.9 million/ml, respectively, *P*=0.0167). Similarly, PLT counts were decreased in B+T compared to BMAC (40.7 ± 30.7 million/ml and 417.5 ± 365.5 million/ml, respectively, *P*<0.0001), however, PLTs were significantly concentrated in BMAC compared to BMA (417.5 ± 365.5 million/ml and 114.8 ± 61.6 million/ml, respectively, *P*=0.0429).

RBC counts were significantly decreased in BMAC and B+T compared to BMA (*P*=0.0322 and *P*<0.0001, respectively). Higher concentration of MSCs were observed in BMAC compared to BMA (0.006% ± 0.01% and 0.00007% ± 0.0001%, respectively, *P*=0.0176). Similar to TNCs and PLTs, the proportion of MSCs significantly decreased in B+T compared to BMAC (B+T: 0.0004% ± 0.001%, *P*=0.0023). Furthermore, patient age and TNC counts did not correlate with MSC concentration (Spearman’s Rank test*, P*=0.3266 and *P*=0.4880, respectively).

Conclusions

BMAC successfully concentrated PLTs, but BMAC preparations were highly variable. Mixing BMAC and thrombin however, as described in the CCR protocol, resulted in a dramatic reduction in TNCs, PLTs and MSCs. TNC counts and patient age could not be used to predict the MSC proportion in the BMAC based on current data. Future work aims to look at the biomolecule profile of BMAC plasma, and to correlate them to patient clinical outcomes.

