functioning hepatocytes. These properties may be useful for the palliative treatment of patients with end-stage liver failure and cirrhosis.

Methods: Five consecutive patients (4 men; mean age 59 years) with the condition were recruited from a medical clinic. Two patients presented with decompensated liver encephalopathy. The aetiologies were viral hepatitis (n=3), alcohol-induced (n=1), and autoimmune/idiopathic (n=1). Liver cirrhosis was confirmed by abdominal ultrasound. Three patients had portal hypertension with splenomegaly. All received umbilical cord-derived mesenchymal stem cells (MSC) via intravenous infusion. Blood samples were taken at baseline, 6 weeks and 3 months after cell treatment and sent for haematology, liver function test and prothrombin time.

Results: All patients tolerated the procedure well. There was generally improvement in all blood parameters at 6 weeks, sustained at 3 months. Specifically two patients with anaemia and thrombocytopenia, presumably due to splenomegaly, demonstrated significant improvement. Hepatitis viral load by PCR also improved significantly in two out of three patients.

Conclusion: MSC infusion improves liver function tests in patients with hepatitis and may potentially play a role in management of end-stage liver failure and cirrhosis. The association between MSC infusion and viral load reduction warrants further investigation.

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THE EFFECT OF ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELS ON B CELL PROLIFERATION AND DIFFERENTIATION

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Background: Mesenchymal stem cells (MSC) have proven immunomodulatory capacity which makes them a promising therapeutic tool in transplantation. While the immunosuppressive effect of MSC on T cell-mediated effector mechanisms has been well studied, less is known about the effects of MSC on B cell-mediated immune responses.

Methods: MSC were isolated from subcutaneous fat tissue from kidney transplant donors. Resting mature B cells from tonsils were obtained by CD43 negative selection with Magnetic Activated Cell Sorting (MACS). MSC were co-cultured with CFSE-labeled B cells stimulated in a T cell-like fashion (anti-IgM + anti-CD40 + IL2) or by PMA/ionomycin activated CD4 T cells. Proliferation and B cell phenotype were analyzed by Flow Cytometry, and IgG production quantified by ELISA.

Results: Proliferation of B cells activated in a T cells-like manner (anti-IgM + anti-CD40 + IL2) was not affected by the presence of MSC, while MSC decrease the proliferation of B cells stimulated with activated T cells. An induction of plasmablasts (CD19+ CD27high CD38high) occurred when B cells were stimulated in a T cell dependent manner or in the presence of activated CD4 T cells. MSC abolished the differentiation into plasmablasts completely, which was correlated with decreased IgG production. Furthermore, MSCs induced an increase in the percentage of CD19+ CD27-CD38high CD24high regulatory-like B cells when stimulated in a Tcell-like fashion.

Conclusion: MSC inhibit B cell differentiation while increasing the proportion of regulatory-like B cells. The reduction of B cell proliferation by MSC is T cell-dependent. These results suggest a therapeutic role of MSC for the treatment of patients suffering from B cell mediated alloreactivity.

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AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STEM CELL TRANSPLANTATION IMPROVES CLINICAL DISABILITY IN PATIENTS WITH ACUTE MIDDLE CEREBRAL ARTERY INFARCT

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Background: Stroke involving the middle cerebral arteries (MCA) confers significant mortality and morbidity due to irreversible neuronal damage. Studies, animal and clinical have shown that bone marrow-derived mesenchymal stem cells (BMMSCs) improve disability in stroke. In this study, we studied the efficacy of autologous BMMSCs in patients with acute MCA infarct.

Methods: In this open-label series, 5 consecutive patients with acute MCA infarct, aged 30-75 years, stroke onset between 2 weeks to 2 months, and National Institutes of Health Stroke Scale (NIHSS) score 10-35 were recruited. Autologous BMMSCs were isolated, expanded in vitro, and infused intravenously. Patients were serially assessed using NIHSS, Barthel Index (BI), Modified Rankin Scale (mRs) at baseline, 3 months, 6 months and 12 months, and magnetic resonance imaging (MRI) at baseline and 12 months.

Results: Mean age of patients was 59 years and mean duration of stroke was 1.1±0.6 months. At baseline the mean NIHSS score was 14.4±2.7, the BI was 31.0±30.1, and the mRs was 4.4±0.6. Following infusion, there were significant improvements in the NIHSS score and BI at 3 months (NIHSS: 7.0±2.6; $p<0.01$, BI: 80.0±18.4; $p<0.01$); NIHSS and BI at 6 months (NIHSS: 5.4±3.2; $p<0.01$, BI: 85.0 ±11.7; $p<0.01$); and NIHSS, BI and mRS at 12 months (NIHSS: 3.0±2.2; $p<0.01$, BI: 91.3±7.5; $p<0.01$, mRs,: 2.0±1.2; $p<0.05$) compared to baseline scores. MRI at 12 months post-BMMSCs treatment showed no significant changes in infarct size compared to baseline. No adverse events were reported.

Conclusion: Our findings suggest that BMMSC infusion is efficacious in reducing clinical disability as early as 3 months with no adverse effects. Autologous BMMSCs infusion appears safe and feasible in improving clinical disability in patients with acute MCA infarct. Future trials involving larger samples are needed to confirm our findings.

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EFFICACY OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS PLUS MESENCHYMAL STEM CELL VERSUS AUTOLOGOUS BONE MARROW MONONUCLEAR CELL ALONE IN ISCHEMIC FOOT ULCER

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Background: Non-healing ischemic foot ulcer has remained an important clinical challenge. Studies have shown that Bone Marrow Mononuclear Cells (BM-MNC) implantation may promote capillary network proliferation while Bone Marrow Mesenchymal Stem Cells (BM-MSC) may promote sturdier arteriolar formation and vascular regeneration. This process of angiogenesis may help resolve ischemic foot ulcers and potentially avoid limb amputation. The purpose of the study was to compare the efficacy of intramuscular implantation of autologous BM-MNC plus BM-MSC versus BM-MNC alone, in ulcer healing of patients with ischemic foot ulcers.

Methods: Seven consecutive patients with non-healing ischemic foot ulcer were recruited. Patients were divided into two groups with 3 patients in BM-MNC plus BM-MSC group (Group A: mean age 40 years, all males; 2 former smokers), and 4 patients in BM-MNC alone group (Group B: mean age 61 years, all female and non-smokers). BM-MNC was injected into the affected leg at baseline (Group A and B) while BM-MSC was injected 4 weeks later (Group A). Ulcer size was measured and recovery of ulcer was recorded at baseline, 1 month and 6 months after the BM-MNC injection.

Results: The baseline mean ulcer size for Groups A and B were 35.6±18.1 cm^2 and 28.1±15.0 cm^2 respectively and mean ankle brachial index (ABI) were 0.66 and 0.76 respectively. In Group A all three patients (baseline ulcer size 28.6 cm^2, 56.1 cm^2 and 22.0 cm^2) reported complete healing of ulcer at 6 months. In Group B, two patients (baseline ulcer size 9.7 cm^2 and 22.7 cm^2) reported complete healing of ulcer at 6 months while another two patients (baseline ulcer size 36.9 cm^2 and 47.6 cm^2)' reported worsening of ulcer.

Conclusion: The combination of BM-MNC and BM-MSC injection is better than BM-MNC alone in promoting ulcer healing. Larger studies involving larger samples are needed to confirm the findings.

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AUTOMATED IMAGE ANALYSIS IS USEFUL FOR SCREENING CELL AGING IN MESENCHYMAL STROMAL CELL CULTURES

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Background: Senescent cells are undesirable in therapeutic cell products due to reduced therapeutic activity and risk of aberrant cellular effects. It has been known for long that early passage mesenchymal stromal cells (MSC) are small and spindle shaped but become enlarged and rounded upon cell aging. However, these changes have not hitherto been quantified. We have developed a screening method based on automated image analysis of cell surface area for identifying senescent MSCs from therapeutic cell cultures.

Methods: MSCs were cultured from bone marrow aspirates to senescence. The cell nuclei and cytoplasm were stained from passage 1, 3, 5, 7 and 8 cells. Fluorescent signals were acquired on different channels using an automated high-throughput microscope and resulting images were analyzed. Several morphometric indices were analyzed for their ability to differentiate between early and late passage cells. Telomere lengths from corresponding cell passages were measured using terminal restriction fragment analysis (TRF) and the expression of cell cycle regulating proteins p16INK4a and p21Cip1/Waf1 was studied by western blot analysis.

Results: The average cell area in passage 1 cells at 14±3 population doublings (PD) was 419 ± 26 µm2. A dramatic increase in the cell surface was evident after passage 3 (25±3 PD), resulting in an average cell size of 2838 ± 292 µm2 at 32±6 PD's. A plateau phase in growth kinetics, telomere shortening and increased p16INK4a expression accompanied the enlargement of the cell surface area. However, even upon aging the cell cultures maintained a small but diminishing population of small cells.

Conclusions: Our findings suggest that MSCs used for cell therapies are of uniform small size at early passages. Automated analysis of the cell surface area may be used as a screening tool of cell aging for therapeutic MSC cultures.

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LARGE NUMBERS OF HUMAN MESENCHYMAL STROMAL CELLS ISOLATED DIRECTLY FROM HUMAN OSSEOUS TISSUES USING GMP COMPLIANT MICROBEAD TECHNOLOGY

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Introduction: Non-expanded, mesenchymal stromal cells (MSCs) represent attractive candidates as therapeutic agents. Avoiding culture-expansion in vitro minimizes manufacturing cost and safety concerns related to potential chromosomal instability associated with culture expanded MSCs. However the use of non-expanded MSCs has been limited due to their low frequency in bone marrow (BM) aspirates the need to concentrate BM derived MSCs and the lack of effective GMP compliant enrichment technologies. Here we evaluated a MSC isolation technology utilising the MSC specific marker CD271.

Methods: Using a clinical grade CD271-conjugated magnetic bead and the CliniMacs cell separation system (Miltenyi Biotec, Germany) we enriched MSCs from three intra-osseous sources. These were whole BM aspirates (n=3, 20ml), discarded irrigation fluids from long bone reaming procedures (RF, n=3) and collagenase-released cell suspensions from discarded osteoarthritic femoral heads (FHs, n=3). The results were analysed by a combination of flow cytometry and colony forming unit fibroblast assay.

Results: MSCs were enriched from all 3 sources; BM, FHs and RF yielded mean $4.0x10^4$ ($3.1\text{-}4.9x10^4$), $1.5x10^6$ ($0.9\text{-}2.4x10^6$) and $2.5x10^5$ ($1.4\text{-}3.8x10^5$) MSCs , respectively, based on measurement of the $CD45^{-/low}CD73^{+}CD271^{bright}$ cell population by flow cytometry, at a purity of 21.8% (4.6-30.8), 55.8% (44.8-69.6) and 35.9% (34.5-36.6), respectively. Enriched suspensions from FH contained on average $2.4x10^5$ colony forming units whereas Enriched RF suspensions yielded on average $1.2x10^4$ colony forming units.

Conclusion: Ready availability of femoral heads from hip replacement surgery and the high yield of MSCs from this tissue makes this technology a viable prospect for allogeneic therapy development. However MSCs isolated from reaming waste fluid represent the best prospect for autologous therapy in the orthopaedic setting.

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COMPARATIVE STUDY OF IMMUNE REGULATORY PROPERTIES OF STEM CELLS DERIVED FROM DIFFERENT TISSUES

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Allogeneic stem cell (SC)-based therapy is a promising tool for the treatment of a range of human degenerative and inflammatory diseases. Many reports highlighted the immune modulatory properties of some SC types, such as mesenchymal stromal cells (MSCs), but a comparative study with SCs of different origin, to assess whether immune regulation is a general SC property, is still lacking. To this aim, we applied highly standardized methods employed for MSC characterization to compare the immunological properties of bone marrow-MSCs, olfactory ectomesenchymal SCs, leptomeningeal SCs, and three different c-Kit-positive SC types, that is, amniotic fluid SCs, cardiac SCs, and lung SCs. We found that all the analyzed human SCs share a common pattern of immunological features, in terms of expression of activation markers ICAM-1, VCAM-1, HLA-ABC, and HLA-DR, modulatory activity toward purified T, B, and NK cells, lower immunogenicity of inflammatory-primed SCs as compared to resting SCs, and indoleamine-2,3-dioxygenase-activation as molecular inhibitory pathways, with some SC type-related peculiarities. Moreover, the SC types analyzed exert an anti-apoptotic effect toward not-activated immune effector cells (IECs). In addition, we found that the inhibitory behavior is not a constitutive property of SCs, but is acquired as a consequence of IEC activation, as previously described for MSCs. Thus, immune regulation is a general property of SCs and the characterization of this phenomenon may be useful for a proper therapeutic use of SCs.