culture and comparatively analyzed them at early and late passages. All of the clones senesced in culture, exhibiting decreased telomerase activity and shortened telomeres. Two clones showed no DNA copy number variations (CNVs) at passage 30. Seven clones had ≥1 CNVs at passage 30 compared with passage 3, and one of these clones appeared trisomic chromosome 10 at the late passage. No tumor developed in immunodeficient mice injected with hUC-MSCs, regardless of whether the cells had CNVs at the late passage. mRNA-Seq analysis indicated that pathways of cell cycle control and DNA damage response were downregulated during in vitro culture in hUC-MSC clones that showed genomic instability, but the same pathways were upregulated in the clones with good genomic stability. These results demonstrated that hUC-MSCs can be cultured for many passages and attain a large number of cells, but most of the cultured hUC-MSCs develop genomic alterations. Although hUC-MSCs with genomic alterations do not undergo malignant transformation, periodic genomic monitoring and donor management focusing on genomic stability are recommended before these cells are used for clinical applications.

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A XENO-FREE CULTURE SYSTEM FOR HMSC FROM VARIOUS SOURCES SUITABLE FOR INITIAL ISOLATION AND EXPANSION TOWARD CLINICAL APPLICATIONS

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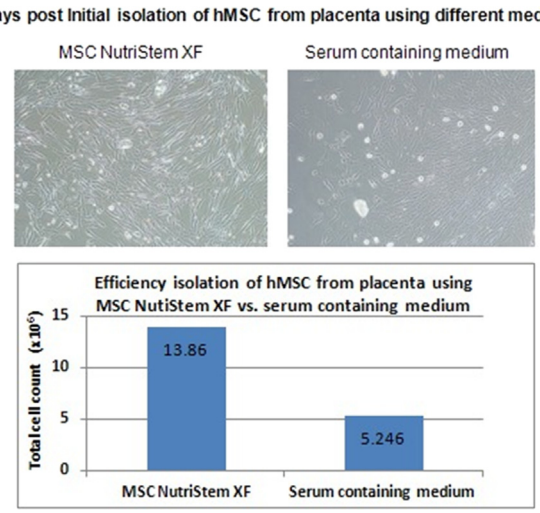
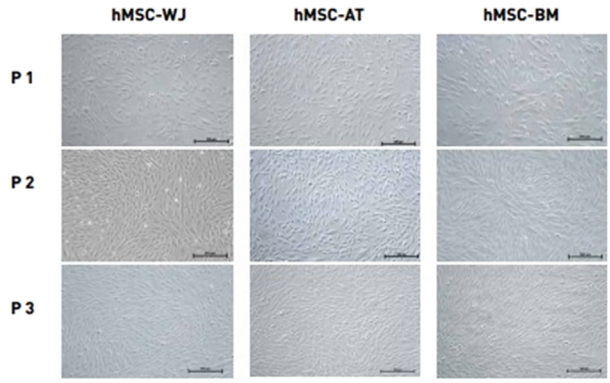
Human mesenchymal stem cells (hMSC) are multipotent adult stem cells present in a variety of tissue niches in the human body. hMSC have advantages over other stem cell types due to the broad variety of their tissue sources, since they are immuno-privileged, and for their ability to specifically migrate to tumors and wounds in vivo. Due to these traits hMSC have become desirable tools in tissue engineering and cell therapy. In most clinical applications, hMSC are expanded in vitro before use. The quality of the culture medium and its performance are particularly crucial with regard to therapeutic applications, since hMSC properties can be significantly affected by medium components and culture conditions. To date, there is no efficient xeno-free (XF) medium for the initial isolation of hMSC from various tissues. In addition, most of the common culture media for growth and expansion of hMSC, as well as auxiliary solutions (for attachment, dissociation, and cryopreservation), are typically supplemented with serum or other xenogenic compounds. A defined serum-free (SF), XF culture system optimized for hMSC isolation and expansion would greatly facilitate the development of robust, clinically acceptable culture process for reproducibly generating quality-assured cells. The present study evaluated a novel XF culture system, comprising MSC NutriStem® XF culture medium and all the required auxiliary solutions for the attachment, dissociation, and cryopreservation of the cells. The system was evaluated for initial isolation of hMSC from various sources, and for long-term culturing under SF, XF culture conditions suited for clinical applications. Results show that the XF culture system for hMSC efficiently supports initial isolation and optimal expansion of hMSC from various sources, while maintaining hMSC features: typical fibroblast-like cell morphology, phenotypic surface marker profile, differentiation capacity, self-renewal potential, and genetic stability.



SF, XF culture system optimized for the Initial Isolation of hMSC



SF, XF culture system suitable for culturing hMSC from various sources

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CULTURE OF INTESTINAL STEM CELLS IN SERUM-FREE CONDITIONS

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Intestinal stem cells (ISCs) are located at the bottom of the intestinal crypts. Additionally, intestinal stem cells have the ability of differentiation into transit amplifying cells which in turn will give rise to all the mature epithelial cells. Recently, intestinal stem cells have taken a great attention as a promising stem cell therapy for many intestinal diseases such as small bowel syndrome. In addition, many challenges were facing the study of ISCs because of the lack of definitive markers and definitive isolation and culture methods. Thus, developing a definitive culture system and serum-free medium of intestinal stem cells will be promising in the clinical applications and may help in the small intestine tissue engineering. In the present study, we are showing ISCs isolation from mouse small intestine. Furthermore, we have selected five significant growth factors which could enhance ISC proliferation in vitro and replace the serum components. Moreover, our optimum growth conditions could maintain the ISC growth in 3D culture and enhanced the enteroid formation ability of the intestinal crypts in matrigel. The results of gene expression analysis of some ISC markers including Lgr5, Bmi1, Ascl2 and PTEN have confirmed that our optimum medium could maintain the stem cell state in this culture system. In a conclusion, these results may help in the enhancement of ISC expansion and understanding the major signaling pathways which maintain the ISC self-renewal and differentiation. In addition, this serum-free medium will be a good tool in the clinical applications.

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UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELL INFUSION IMPROVES LIVER FUNCTION IN LIVER CIRRHOSIS AND IS ASSOCIATED WITH VIRAL LOAD REDUCTION

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Background: Mesenchymal stromal cells (MSC) may attenuate inflammation and T-cell mediated injury. MSC has also been proven to differentiate into

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.