ABSTRACTS

Oral Abstracts

1

DATA IN SUPPORT OF THE CLINICAL USE OF ADIPOSE DERIVED MSC: GROWTH, STORAGE, FUNCTION AND SAFETY

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Mesenchymal stromal cells (MSC) are a promising treatment for autoimmune disorders and tissue regeneration. Our laboratory has focused on an autologous approach using adipose tissue (AT) derived MSC (adMSC). In an effort to move these therapeutic cells into the clinic, we have performed studies to optimize and characterize the processes and the cells as the foundation of the manufacturing protocols for our clinical trials. Early on, we developed a media supplement derived from human platelets that is the basis of our culture protocol (PLTMax, Mill Creek Lifesciences, Rochester, MN). We have used this protocol to grow adMSC from otherwise healthy patients undergoing bariatric surgeries and from >30 patients with a variety of diseases including ALS, Crohn's disease, type 1 diabetes, and ovarian cancer. We saw no differences in growth kinetics or phenotype associated with underlying disease. We have successfully cultured adMSC to more than 25 population doublings without loss of growth rate or change in phenotype. This protocol typically yielded 1×10^9 MSC in 3 weeks from a starting product of 1-2 gms of AT. When thawed, cells frozen for more than a year retained their pre-freeze proliferation rate. adMSC inhibited dendritic cell maturation as well as inhibited dendritic cell mediated stimulation of CD4 T cells. Safety studies in rabbits, pigs and mice have shown that cells have expected bio-distribution. No malignant transformation was seen during the injection of more than one billion adMSC into immune deficient mice. These and other preliminary data have led to the opening of phase I clinical trials using these cells to treat ALS, multiple system atrophy, and renal stenosis. Two other applications are under review.

2

A TISSUE ENGINEERING CONSTRUCT COMPRISING HUMAN ENDOMETRIAL MESENCHYMAL STROMAL CELLS AND POLYAMIDE/GELATIN MESH EVALUATED IN A PRECLINICAL ANIMAL MODEL OF PELVIC ORGAN PROLAPSE REPAIR

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Pelvic organ prolapse (POP) is the herniation of bladder, bowel and/or uterus into the vagina causing urinary incontinence and sexual dysfunction. POP results from childbirth injury. 11-19% of women will be treated for POP by reconstructive surgery with or without polypropylene mesh. Complication rates approach 30%. Our aim was to use a tissue engineering approach to determine whether human endometrial stromal cells (eMSC) purified from the regenerative uterine lining improves the performance of a composite polyamide/gelatin mesh in a rat model of wound repair.

eMSC were isolated from hysterectomy tissue using W5C5-labelled magnetic beads, cultured to P6 and labelled with Vybrant® DiO. Warp-knitted polyamide monofilament meshes were coated with 12% cross-linked gelatin, gamma-sterilized and coated with fibronectin. eMSC (250,000 cells) were seeded onto 25 × 10 mm meshes and implanted subcutaneously in the dorsum of immunocompromised nude rats for 7, 30, 60, 90 days (n = 8/group). Controls were meshes without cells. Explanted samples were analysed by flow cytometry to detect DiO-labelled cells, by immunohistochemistry to assess foreign body reaction and tissue integration. Collagen III/I ratios were quantified by chemical assays, collagen organisation by birefringence and tensile testing by Instron.

Implanted meshes were well tolerated. eMSC were detected in explants until 14 days post-implant. eMSC/meshes contained significantly fewer CD45+ leukocytes, CD68+ macrophages at 90 days, significantly increased M1/M2 macrophage ratio and neovascularisation at 7 days than controls (all $P < 0.05$). New collagen was observed in meshes with and without eMSC. By 90 days, there were improved biomechanical properties of eMSC/mesh, increased collagen type III/I ratios and more collagen organisation than controls.

This tissue engineering approach, using eMSC delivered on polyamide/gelatin composite mesh, reduced inflammatory cells around implanted mesh fibres, promoted neovascularisation and improved mesh distensibilty over time, suggesting that this might be an alternative novel approach for future treatment of POP.

3

INTRA ARTICULAR INJECTION OF AUTOLOGOUS BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS IN PATIENTS WITH MODERATE TO SEVERE OSTEOARTHRITIS

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Background: Bone marrow-derived mesenchymal stromal cells (BMMSC) can be expanded ex vivo which have the ability to regenerate cartilage for accelerated healing of the knee as demonstrated by animal studies and early clinical reports. In this study we have evaluated the safety and feasibility of using autologous BMMSC as an intra-articular injection for the treatment of symptomatic moderate to severe osteoarthritis.

Methods: Fifteen patients with symptomatic moderate to severe knee osteoarthritis were recruited. All patients have persistent non-improving or deteriorating pain despite regular oral analgesics and multiple hyaluronic injections. Autologous BMMSC was resuspended in a mixture of hyaluronic acid and autologous platelet rich plasma before intra-articular injection procedure. Patients were assessed and followed-up using the Oxford Knee Score (OKS) and magnetic resonance imaging (MRI) for up to 12 months.

Results: The mean OKS at 6 and 12 months after BMMSC injection increased significantly (42.6 ± 6.2 and 44.8 ± 8.1) when compared to baseline scores (35.2 ± 6.5). At 12 months, an improvement of OKS of 4 points or more was observed in 10 patients when compare to their baseline scores for both knees while 2 patients experienced improvement in the right knee only. MRI at 12 months post-BMMSC treatment showed noticeable improvement in 60% of patients including mean increase in cartilage thickness from baseline, resolution of subchondral cysts and reduction of effusion.

Conclusion: Autologous BMMSC injection is safe, feasible and may be beneficial for the symptomatic treatment of patients with moderate to severe osteoarthritis who have failed conventional treatment.

4

EXTRACELLULAR MATRIX FREE MICROCARRIER CULTURES OF HUMAN PLURIPOTENT STEM CELLS INDUCED BY INHIBITION OF ROCK-MYOSIN II SIGNALING

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Large quantities of human pluripotent stem cells (hPSC) needed for therapeutic applications can be obtained in scalable suspended microcarrier cultures. However, these microcarriers have to coated with animal or human extracellular matrix (ECM) proteins which can present safety risks, and/or are very expensive for large scale use. This study demonstrates that human embryonic stem cells (HES-3, H7) and induced pluripotent stem cell (IMR90) can be propagated on non-coated positively charged cellulose microcarriers in serum free medium containing ROCK inhibitor, (Y27632) or myosin inhibitor, Blebbistatin. Dephosphorylation of myosin phosphotase 1 (MYPT1) and myosin light chain 2 (MLC2) were observed in the presence of these two inhibitors suggesting that reduced myosin contractility is responsible for hPSC survival and growth on ECM-coating free microcarriers. Cells were propagated on the non-coated microcarriers for at least 15 passages while maintaining pluripotency and karyotype stability. Scalability of this platform was demonstrated in 100 ml spinner flask resulting in cell yields of 2.3 × 106 cells/ml (HES-3) after 5 days of growth. The capability of these cells to differentiate into the three primary lineages was demonstrated in in-vitro embryoid bodies and in-vivo teratoma formation studies. Moreover, directed differentiation to PSA-NCAM+ neural progenitor cells was demonstrated, high cell yields ($9.1 \pm 1.2 \times 106$ cells/ml) and expression levels ($91 \pm 1.1\%$ cells expressing polysialylated neuronal cell adhesion molecule (PSA-NCAM)) were obtained. This defined serum- and coating- free scalable microcarrier culturing system