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Mesenchymal cells are multipotential and able to self-renew and to differentiate into different lineages. During this stage, they express embryonic stem cells markers as SOX-2, Nanog and Oct-3/4. The aim of this study was to test these transcription factors in our Mesenchymal Adipose-Derived Stem cells (MADS).

MADS were isolated from rat inguinal fat and cultured in Amniomax® (Gibco) during more than 180° passages. We analyzed the immunohistochemical expression of the different markers: Nanog, Oct-3/4 and SOX-2. Proliferation studies were performed in first passage cells during 15 days by flow cytometry.

The three markers appeared during all the passages. The average percentage of Oct-3/4 was about a 60%; SOX-2, 45%; and Nanog, 27%. Cells from first passage showed a high proliferation rate 206 times higher than the initial population.

If cells isolated from rat adipose tissue and cultured in Amniomax are able to survive for more than 175 passages, showing a high proliferation rate and the expression of embryonic stem cells markers in a part of the population during all the passages, they can represent a stable culture source to be employed in tissue engineering.

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(268) Mesenchymal Stem Cell Proliferation and Metabolic Profiling in Spinner Flasks

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Proliferation of Human Bone Marrow Mesenchymal Stem Cells (HBMSC) in 2D systems is not optimal for achieving high cell numbers (e.g. 200–800 million cells). In order to expand HBMSC to significant numbers in a closed and controlled environment, we are developing a bioreactor system involving a spinner flask containing microcarriers.

We tested different stirring rates and feeding regimes with respect to viable cells. In addition, we obtained a metabolic profile including glucose, lactate, glutamine and ammonia for HBMSC under the different conditions mentioned above.

Our data suggests that in the range of stirring rates used (10–30 RPM), there are little differences in growth rate, metabolite consumption and production. Independent of the stirring rate, the data shows that HBMSC enter a stationary phase after a few days in culture. By adding 30% working volume of microcarriers and medium, HBMSC did not enter a stationary phase and proliferated linearly for 14 days.

Interestingly, HBMSC proliferation rate continues to be higher under static than under dynamic conditions. This is reflected by the HBMSC growth rates (μ) in the two systems: 0.04 hr^{-1} in T-flasks and 0.01 hr^{-1} in spinner flasks.

We are developing a mass balance model from metabolite and viable cell concentration measurements obtained. Matlab® functions allow us to determine quantitative differences on growth profiles. From the model, we expect to obtain the growth kinetics and metabolic rates of HBMSC under different conditions.

(269) Mesenchymal Stem Cells and Tensile Strain

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Human mesenchymal stem cells (MSCs) have potential applications in tissue engineering because they are multipotent, unspecialized cells which can differentiate into numerous different lineages. Evidence indicates that tensile strain can have multiple effects on cell responses, including proliferation, alignment and gene expression. However, there is limited data describing its affect on MSCs. We investigated the effects of tensile strain application on gene expression in primary human MSCs.

MSCs (Lonza) were grown in monolayer on pronectin-coated 6-well tissue culture plates (Flexcell). Cells were cultured at 5×10^4 cells/well in DMEM supplemented with 10% FCS, 1% L-glutamine and 1% antibiotics. Uniaxial tensile strain, equivalent to 1 and 3% cell elongation, was applied to cells for 1 hour using a FX-4000T system (Flexcell), and samples were taken 2 hours subsequently. Isolated total RNA samples were used for microarray analysis using Affymetrix Human Genome U133 Plus 2.0 arrays (performed by Almac Diagnostics). Real-time PCR was performed to validate genes of interest identified through microarray analysis.

Microarray analysis revealed >250 genes were differentially expressed compared to control, for both 1 and 3% cell elongation. In each case, approximately 25% of the genes were up-regulated. In both conditions, expression of aurora kinase A interacting protein 1 (a negative regulator of mitosis) was up-regulated the greatest, approximately 5-fold. Several genes relating to actin binding were also found to be differentially expressed in both conditions.

Our data indicate that MSCs exposed to tensile strain have altered gene expression profiles that indicate a transition away from proliferation.

(270) Mesenchymal Stem Cells as Substitutes for Schwann Cells

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Schwann cells (SCs) provide physical support and guidance for peripheral nerve regeneration following injury. *In vitro* these supporting cells are slow growing, hence not well suited to a tissue engineering approach to nerve repair. Adult rat bone marrow mesenchymal stem cells (MSCs) were differentiated into SC-like cells using an established cocktail of growth factors: glial growth factor-2, basic fibroblast growth factor and platelet derived growth factor.