Scale-up and Manufacturing of a Mesenchymal Stem Cell (MSC) Therapy for the Treatment of Graft-versus-Host Disease (GvHD)

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Introduction

Mesenchymal stem cells (MSCs) are an important tool in regenerative medicine and tissue engineering and are the focus of intense research towards clinical applications. For instance, acute steroid-refractory Graft-versus-Host Disease (GvHD) has been shown to be responsive to treatment with mesenchymal stem cells (MSCs) by several clinical studies. However, clinical expansion of these cells in culture environments with human or animal origin reagents such as media and/or extracellular matrix protein (ECM)-coated surfaces requires expensive and time consuming testing for lot-to-lot variability and adventitious agents. Animal-free (defined as xeno-free and human origin components-free) culture environments significantly reduce this risk. In this study human bone marrow-derived MSCs, cultured on a synthetic Fibronectin ECM mimetic surface in xeno-free medium, were expanded for treatment targeting GvHD. Production of cells for *in vitro* studies and pre-clinical development was accomplished through process scale up beginning in T-flask formats and transitioning to large closed system stacked cell culture vessels. Cells maintained their characteristic morphology and immunophenotyping (positive for CD13, CD29, CD44, CD73, CD90, CD105, and negative for CD45, CD80, and HLA-DR) and achieved comparable growth characteristics such as population doubling time and yield. Additionally, two other cell types, human glioblastoma (A172) cells and hamster kidney (BHK-21) fibroblasts, were cultured on the Fibronectin peptide-coated synthetic surface in both flask and stack vessels. Cell attachment, growth and morphology on ECM mimetic flask surfaces was demonstrated to be comparable to cells grown on the larger scale stack coated surfaces. These Fibronectin peptide-coated closed system vessels provide a xeno-free cell expansion system enabling scale up options for clinical research.

Materials and Methods

MSC Culture and Characterization

Collection, isolation, and expansion of hMSCs from bone marrow: Human bone marrow (BM) aspirates were obtained from healthy donors (age 20 to 40 years old) (Lonza) after informed consent. BM mononuclear cells (MNC) were isolated, purified, and plated. After 3 days of incubation non-adherent cells were discarded, and the adherent culture plated on Corning® PureCoat™ ECM Mimetic Fibronectin peptide (Fn-mimetic) T-flasks and Corning CellSTACK® vessels. MSCs used in this study were between P2 to P6.

Immunophenotype characterization was assessed by flow cytometry using a BD FACSCalibur™ and analysed using Flowing Software 2. Analysed antibodies are listed, appropriate isotype controls were used.

Viability and yield: Cell number and viability were determined using the Trypan Blue (Gibco) exclusion method.

Morphological analysis: At 80% confluency, cells were stained with rhodamin phalloidin followed by DAPI (Molecular Probes). Images were acquired using an optical fluorescence microscope (Axiovert 200M, Zeiss).

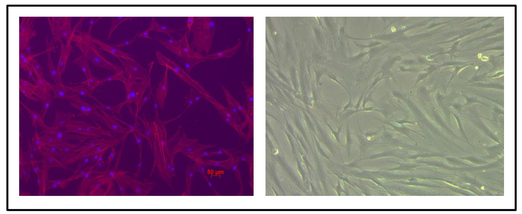
Multilineage differentiation: MSCs expanded on the PureCoat ECM mimetic cultureware Fibronectin peptide surface were visually studied for the ability to undergo multi-lineage differentiation following accepted protocols. Cells were stained with Oil Red-O (Sigma, Adipogenic), von kossa (Osteogenic), and Alcian Blue (Sigma, Chondrogenic).

Culture of other cell types

A172 cell culture: Cells were cultured in low glucose DMEM (ThermoFisher) supplemented with 10% FBS (GE Healthcare). Cells were suspended in low glucose DMEM with 0.2 mg/mL BSA and seeded onto PureCoat ECM mimetic Fibronectin peptide 2-layer Corning CellSTACK and T-75 flasks at 26,000 cell/cm². Vessels were incubated in 37°C/5% CO2 atmosphere for 18 hours. **BHK-21 cell culture:** Cells were cultured in low glucose DMEM supplemented with 10% FBS (ThermoFisher). Cells were suspended in low glucose DMEM with 1 mg/mL BSA and seeded onto PureCoat ECM mimetic Fibronectin peptide 2-layer CellSTACK and T-75 flasks at 35,000 cell/cm². Vessels were incubated in 37°C/5% CO_2 atmosphere for 18 hours.

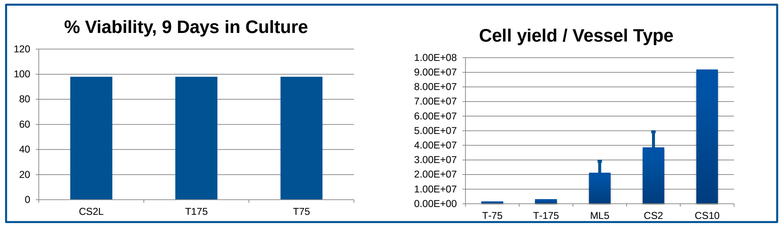
Image analysis: After 18 hours, cells were examined and photographed at 10X magnification at multiple locations. Image segmentation tools using ImageJ software (NIH) was utilized to calculate cell attachment expressed as % cell confluence.

Figure 1. MSC Culture



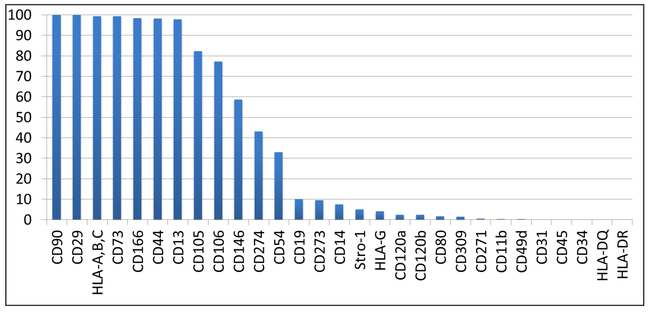
Typical MSC morphology was observed for the MSCs cultured on Fn-mimetic surface.

Figure 2. Viability and cell yields



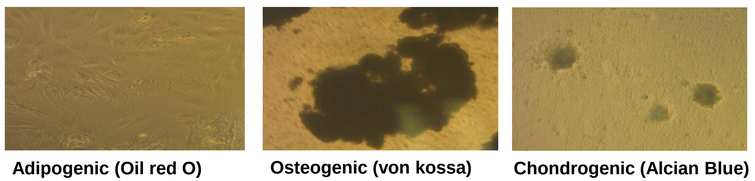
Viability of MSCs cultured on Fn-mimetic Corning CellSTACK 2-layer, T-175, and T-75 flasks were comparable. Cell yields from T-75, T-175, 5 layer Multi-Flask(n=3), CellSTACK 2 (n=3) and 10 layer demonstrated scalable yields.

Figure 3. Immunophenotyping



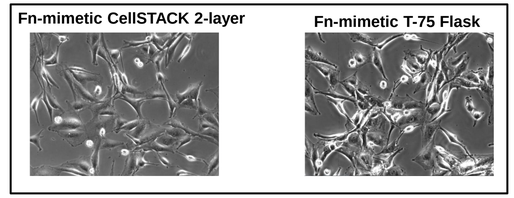
MSCs were positive for CD13, CD29, CD44, CD73, CD90, CD105, and negative for CD45, CD80, and HLA-DR.

Figure 4. Multi-lineage differentiation



MSCs were differentiated into adipogenic, osteogenic, and chondrogenic lineages.

Figure 5. Culture of Other Cell Types – A172 culture



Cell confluence (%) for A172 cells cultured on Fn-mimetic Corning CellSTACK 2-layer and T-75 flasks were comparable.

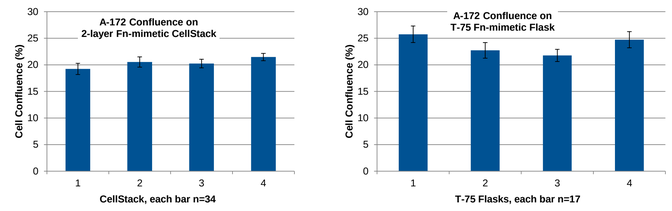
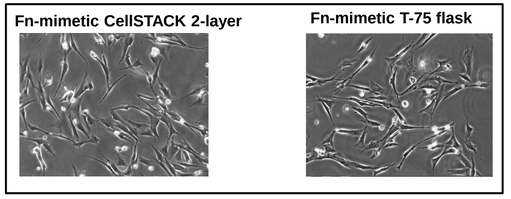
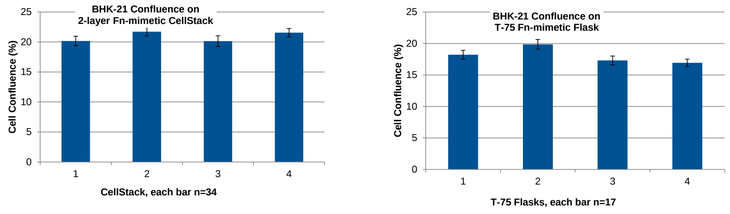


Figure 6. BHK-21 culture



Cell confluence (%) for BHK-21 cells cultured on Fn-mimetic Corning CellSTACK 2-layer and T-75 flasks were comparable.



Conclusion

Corning PureCoat ECM mimetics cultureware Fibronectin peptide-coated vessels provide a xeno-free cell expansion system enabling scale up options for clinical research.

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