A Standardized and Well-Characterized MSC Source for Rapid Biofabrication & Translational Research

Lye Theng Lock, PhD; Iain Farrance, PhD; Priya Baraniak, PhD; Ang-Chen Tsai; Yijun Liu; Teng Ma, PhD; Jon Rowley, PhD
1. RoosterBio, Inc. 4539 Metropolitan Court, Frederick, MD 21704; 2. Florida State University, 2525 Pottsdam Street, Tallahassee, FL 32310

ABSTRACT

Human bone marrow-derived Mesenchymal Stem Cells, or hMSCs, have been recognized as potential patient-specific drugstores, and are a key raw material for future therapeutic, engineered tissues, and medical devices. Today’s commercially available hMSCs for research and product development are expanded in different culture environment and used at varying population doubling level (PDL). This inconsistencies result in experimental procedures that are fraught with variability and studies that are non-reproducible. We have taken a bioprocess engineering approach to generate a standardized and well characterized hMSC source. By utilizing consistent and scalable bioprocesses, high volumes of hMSCs were produced at a much affordable cost. We further characterized hMSCs generated from 3 different donors at a consistent PDL of 11-13 for their cell surface markers expression (CD73, 90, 105, and 166) and angiogenic cytokines secretion levels (VEGF, IL8, TIMP1, TIMP2, FGF, HGF), inducible immunomodulatory function (inducible IDO expression), as well as their differentiation potential into osteo-, adipo- and chondrocytes. Our study shows that hMSCs isolated and expanded using standardized bioprocessing techniques have minimum donor-to-donor variability in terms of their doubling time (Te = 33.5±0.8 for n=4 donors) as well as their cell characteristics. We believe that these standardized and affordable off-the-shelf hMSCs will benefit product developers and translational researchers utilizing hMSC technology for biotissue and product development efforts.

MULTIPOTENT MSC

Fig. 1: (A) MSCs with the potential for self-renewal and the ability to differentiate to cells from a number of mesenchymal lineages including fat, bone, cartilage, and muscle both in vitro and in vivo is a promising source for tissue engineering application (B) Expansion profile of RoosterBio hMSCs (Lots A and B) compared to typical MSC culture in 10% FBS + DMEM show accelerated cell expansion (10-fold increase in cell yield within 4-5 days vs. 20 days for MSCs in conventional culture media).

MSC MULTILINEAGE POTENTIAL

Fig. 2: Expanded multipotent RoosterBio MSCs differentiate into (A) adipocytes (evidenced by oil red o staining of lipid vacuoles), (B) osteocytes (evidenced by Alizarin red and (C) chondrocytes (evidenced by toluidine blue staining).

IMMUNOMODULATORY RESPONSE

Fig. 3: Expanded MSCs were >95% positive for stromal cell surface marker expression (CD73, 90, 105, and 166) and did not express (>5% positive) non-stromal surface markers (CD14, CD34 and CD45).

ECONOMIC ANALYSIS FOR CREATING MSC SPHEROIDS

Fig. 4: MSCs secreted clinically-relevant angiogenic cytokines (FGF, HGF, IL8, TIMP1, TIMP2, and VEGF) 5 days post-expansion in RoosterBio media.

FUTURE WORK

- Spheroids tri-lineage differentiation
- Spheroids in hydrogel encapsulation
- Spheroids molding & bioprinting