Scalable bioreactor-based production of human Mesenchymal Stem Cell (hMSC) 3D aggregates using microcarriers with thermo-reversible surfaces

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Mesenchymal Stem/Stromal Cells, or MSCs, will be key components of future therapeutics, engineered tissues, and medical devices. Recently, exciting research has shown that culture of MSCs as 3D aggregates (3D-hMSC) improves biological activity over MSCs grown as a monolayer. These advantages include greater differentiation, increased paracrine factor secretion, enhanced immunomodulatory activity, resistance to ischemia, smaller cell size, and therefore, improved pre-clinical results. Additionally, in tissue engineering applications, 3D-hMSC facilitate the assembly of microtissues. Clinical and tissue engineering application of 3D-hMSC require large cell numbers (100M to 10B cells). However, production techniques for 3D-hMSC are currently not scalable to levels consistent with industrial needs. For 3D-hMSC use to become widespread, 3D-hMSC production processes must be scalable, cost effective, and clinically-compatible, and be capable of generating billions of consistent, highly functional 3D-hMSC within a single manufacturing lot. Here we report on a scalable production technology for 3D-hMSC. hBM-MSC were expanded on microcarriers with thermo-reversible surfaces (TRM) and 3D-hMSC produced directly by temperature-dependent release of mini-cell sheets from the TRM. To test this scalable method, we hypothesized that hMSCs can be expanded on TRM in bioreactors and that 3D-hMSC with enhanced function, similar to AggreWell 3D-hMSC, can be produced by this method. hBM-MSC grew efficiently on the TRM and formed 3D-hMSC upon thermal release. We compared 3D-hMSC produced on the TRM (Bioreactor aggregates, BR-Aggs) to 3D-hMSC produced in AggreWells (Traditional aggregates, TR-Aggs) in assays for cell viability, immunomodulatory potential (IFN-gamma induced indoleamine 2,3-dioxygenase (IDO) upregulation), and angiogenic cytokine secretion. BR-Aggs and TR-Aggs were similar, with high cell viability, activation of IDO under basal conditions and further induction by IFN-gamma treatment, and displayed comparable angiogenic cytokine secretion levels (VEGF, HGF, TIMP-1 and -2, FGF2, and IL-8). These results confirm the hypothesis that the scalable TRM technology can be used to produce high-quality 3D-hMSC for translational researchers in Regenerative Medicine and Tissue Engineering.

The work reported in this abstract was supported by an SBIR award to JR from the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R43HL128698. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.