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.

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