Annals of Stem Cell Research & Therapy

Editorial
Published: 26 Apr, 2018

Mesenchymal Stem Cell Spheroids Therapy

Hu Zhang* and Jiabin Zhang

Department of Biochemical Engineering, The University of Adelaide, Australia

Editorial

Mesenchymal Stem Cells (MSCs) hold great promise in treating degenerated tissues or organs because they are capable of multi-lineage differentiation and self-renewal. MSCs are typically expanded *in vitro* on the fibronectin-coated polystyrene surface in a two-dimensional (2D) format. However, the 2D *in vitro* expansion often is associated with loss of differentiation capacity, replicative cell senescence, and reduced paracrine production. MSCs have been clinically trialled as single cell suspensions. Unfortunately, above 99% of MSCs locally administrated to injured hearts are cleared after 4 days of administration, and if MSCs are systematically injected, more than 85% MSCs disappeared in precapillaries due to vascular obstructions. It is also noted that engraftment of MSCs into the injured sites is rarely observed, which may be due to hypoxia, inadequate nutrient supply or inflammatory response within the target sites. The therapeutic efficacy of transplanted MSCs is believed to be correlated with the paracrine secretions from MSCs in response to micro environmental and hormonal signals, which enhance tissue repair or prevent tissue degeneration. One strategy of improving the therapeutic potential of MSCs clinically is to apply multicellular spheroids of MSCs. The spheroids are achieved by hanging drop, low attachment surface, forced aggregation, membrane-based surface, or scaffold-assisted techniques, and the detailed analysis of these techniques can be found in our recent review paper [1]. We have developed a microgel-assisted technique for spheroid formation, and this takes 24 hours to form aggregates of around 100 microns from human primary MSCs. Most importantly, this approach generates very uniform-sized spheroids with a much narrower size distribution in comparison with low attachment surface culture. The spheroids formation in 3D is driven by a self-assembly process and cells experience a physiologically relevant external environment. The spheroid structure has a core consisting of tightly compacted cells adherent to each other, and an extra layer of cells surrounding the core. This spheroid structure allows establishment of heterogeneous spatial distribution pattern of oxygen, nutrients, metabolites, and signalling molecules. This structure also offers better cell-to-cell contact and Cell-to-Extracellular Matrix (ECM) contact than 2D cell culture and these contacts are essential for maintaining intracellular functions. MSC spheroids have remarkable biological properties, such as marked anti-inflammatory effect, enhanced angiogenesis, and augmented tissue regenerative and reparative functions with enhanced stemness and improved cellular viability. It has been confirmed that these enhanced biological functions are directly related to up regulation of gene expression associated with hypoxia, angiogenesis, inflammation, stress response, and redox signalling. MSC spheroids have enhanced differentiation capacity that is evidenced by markedly improved differentiation into multi-lineages (chondrogenic, osteogenic, adipogenic, neurogenic, hepatogenic lineages), delayed cell senescent process, increased up regulation of pluripotency marker genes (NANOG, SOX2, POU5F1/OCT4), and enhanced colony formation capacity in comparison with cells from monolayer culture. We have demonstrated that MSC spheroids formed in the thermo sensitive microgels have significantly up-regulated messenger RNA expression of chondrogenic and osteogenic genes even in the absence of induction media on day 9. In the presence of induction media, mRNA expressions of chondrogenic, osteogenic and adipogenic genes of MSC spheroids are significantly higher than those in the pellet and 2D cultures [2]. The possible underlying molecular mechanisms for enhanced differentiation capacity include activation of Rho/Rho-associated kinase pathway to facilitate intercellular communication; inducing intracellular calcium signalling for increased gene expression of N-cadherin and WNT proteins for cell-to-cell contact; and hypoxia-associated signalling cascades. Augmented tissue regenerative capacity is also ascribed to increased post transplanted MSC cell viability and enhanced proliferation ability as spheroids express up regulated anti-apoptotic Bcl-2 and down regulated proapoptoticBax.

Inflammation is adaptive response to tissue damage, including hemostasis and recruitment of inflammatory cells. MSC spheroids have found to be able to suppress inflammation and reduce acute kidney injury. Cells within the spheroid structure up regulate pro-inflammatory cytokines (IL1A, IL1B and IL8), inflammatory modulating cytokines (TSG6, LIF, STC-1, IL1RN), chemokines

OPEN ACCESS

Correspondence:
Hu Zhang, Department of Biochemical Engineering, The University of Adelaide, SA 5005, Australia, Tel: +61 8 8313 3810;
E-mail: hu.zhang@adelaide.edu.du

Received Date: *16 Mar 2018*
Accepted Date: *23 Apr 2018*
Published Date: *26 Apr 2018*

Citation:
Zhang H, Zhang J. Mesenchymal Stem Cell Spheroids Therapy. Ann Stem Cell Res Ther. 2018; 2(2): 1015.

(chemokine ligand 2 and chemokine ligand 7) for recruiting inflammatory cells. The autocrined pro-inflammatory cytokines from MSC spheroids as molecular switches convert liposaccharide-stimulated macrophages from a primarily pro-inflammatory M1 phenotype into more anti-inflammatory M2 phenotype. Single cell suspensions after intravenous injection also form microemboli-like cell aggregates in the lung, and the cell aggregation activates secretions of anti-inflammatory factors, such as Tumour Necrosis Factor (TNF) 1-alpha and TNF-stimulated protein 6 after 12-24 hours of entrapment. This finding triggers exploring of MSC spheroids for therapeutic applications. High angiogenic and vasculogenic potential of MSC spheroids grants the structure as a building block for new tissue constructs. Angiogenesis is essential for nutrients/molecules transport into and wastes out of tissues. For spheroids above 100 microns, pronounced up regulation of angiogenic and anti-apoptotic factors is found in the spheroid structure, more than 100 folds higher than those from monolayer culture, including hypoxia-induced survival factors, vascular endothelial growth factor, and stromal cell-derived factor. These cytokines produced from MSC spheroids are used for paracrine stimulation of angiogenesis. These factors also facilitate differentiation of MSCs into endothelial cells and formation of micro vessels. Paracrine stimulation of angiogenesis and differentiation of MSCs into endothelial cells have been explored for vascularization of *in vitro* tissue constructs. Meanwhile, stromal cell-derived factor, hypoxia-regulated small MW chemokine, allows homing of circulating CXC-receptor positive endothelial cells to ischemic tissues to develop micro-vasculature. We embed cardiac stem cells into thermos-responsive nanogels to form multicellular spheroids, and angiogenic factors are produced to induce growth of cardiomyocytes and regeneration of heart tissues [3]. MSC spheroids have great therapeutic potential, while the underlying mechanisms are not clear. More extensive and in-depth research is required before MSCs spheroids enter into clinical trials. Fabrication of MSC spheroids with a uniform size is also a challenge although robot-assisted and microgel-facilitated production platform is explored. The compositions of MSCs spheroids are tail or able through co-culture of different cell types for specific application purposes, but culture conditions may compromise biological functions of MSC spheroids. Building of macrotissues for implantation or drug evaluation is possible by aligning and fusing MSC spheroids into an integrated structure, and more advanced technologies such as bioprinting and laser guided patterning are required to adapt for MSC spheroid applications.

References

1. Cui X, Hartanto Y, Zhang H. Advances in multicellular spheroids formation. J Royal Soc Interface. 2017.
2. Zhang J, Yun S, Bi J, Dai S, Du Y, Zannettino A, et al. Enhanced Multi-Lineage Differentiation of Human Mesenchymal Stem/Stromal Cells within Poly (N-isopropylacrylamide-Acrylic Acid) Microgel-Formed Three-Dimensional Constructs. J Mater Chem B. 2018:12.
3. Tang J, Cui X, Caranasos TG, Hensley TM, Vandergriff AC, Hartanto Y, et al. Heart Repair Using Nanogel-Encapsulated Human Cardiac Stem Cells in Mice and Pigs with Myocardial Infarction. ACS Nano. 2017;11(10):9738-49.