Soft Hydrogel as Cell Culture Substrate to Direct MSCs Proangiogenic Potential through ROS-NRF2-HIF Signaling

Haibo Yang [1], Melissa Kao Hui Lee [2], Chor Yong Tay [1,2].
[1] School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, S(639798)
[2] School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, S(637551)

Statement of Purpose:

Over the past two years, there has been a discussion whether mesenchymal stem cells (MSCs) should be renamed as "medicinal signaling cells". [1] This is an outcome of a paradigm shift in the understanding the role of MSCs in regenerative therapy, in which the paracrine signaling and cell-cell communications are the main approaches for the MSCs to exert their healing effects rather than relying on the engraftment and differentiation.

Recent studies have shown that the signaling molecules secreted by MSCs (i.e. secretome) contain a broad repertoire of proteins, which are beneficial in treatments of cardiovascular disease, neurological disorders and kidney damage. [2] In this particular study, we have successfully demonstrated that the material properties (i.e. stiffness) were able to induce the secretion of proangiogenic secretome, together with the underlying biological mechanism and the potential biomedical applications.

Methods:

Polyacrylamide (PA) hydrogel was prepared on γ-methacryloxypropyltrimethoxysilane treated coverslip with a ratio of 4:0.15 for acrylamide/bis-acrylamide. Subsequently, the hydrogel surface was activated with Sulfo-SANPAH followed by coating with fibronectin and used as soft substrate for MSCs culture. As control, fibronectin was coated onto coverslip directly and used as hard substrate. MSCs was then seeded on both soft and hard substrates in complete growth medium for overnight, and subsequently cultured in serum free DMEM (SFM) for 48h before cell signaling study and secretome collection. The intracellular oxidative stress was characterized by CellROX™ Orange staining, while intranuclear translocation of NRF2 and HIF1α was examined using corresponding antibodies. The expression of VEGF and bFBF by MSCs were assessed by qPCR analysis. The secretome collected from MSCs were further used as supplemental growth factors to investigate the proliferation and angiogenesis of endothelial cells (HUVECs). Briefly, the secretome was mixed with EndoGRO medium at a ratio of 30% to 70%, while a mixture of SFM and SCME001 was used as control. After 48h of culture, the proliferation of HUVECs was characterized by alamarBlue® viability assay. Matrigel and μ-Plate Angiogenesis 96 well were also used to evaluate the effect of secretome on the tube formation capability HUVECs, which was then analyzed using Angiogenesis Analyzer from ImageJ. Statistical analysis was done using one way ANOVA in SPSS, with significance level of 0.05.

Results:

Soft substrate was found to induce the intracellular ROS expression of MSCs, which was then confirmed by the activation of NRF2 signaling that correspondence to the oxidative stress via intranuclear translocation. As a result, HIF1α was stabilized and translocated to the nuclear of MSCs cultured on soft substrate (Figure 1).

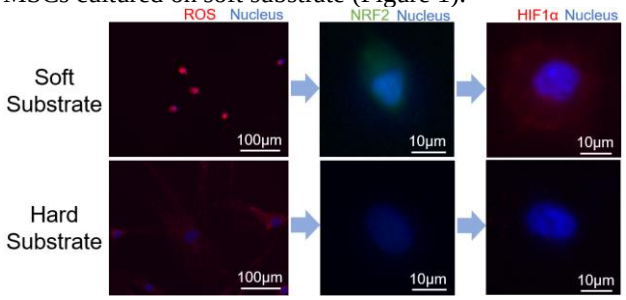

Figure 1. ROS staining, NRF2 staining and HIF1α staining of MSCs cultured on soft and hard substrate.

HIF signaling is a crucial regulator of angiogenesis genes. Hence, activation of HIF signaling induced the up-regulation of VEGF and bFGF expression of MSCs cultured on soft substrate, which are the well-known pro-angiogenic proteins (Figure 2a). Further studies showed that these secretome from MSCs (i.e. soft substrate) successfully used as supplemental growth factors in promoting the proliferation (Figure 2b) and the tube formation capability of HUVECs (Figure 2c), which is significantly higher as compared to hard substrate.

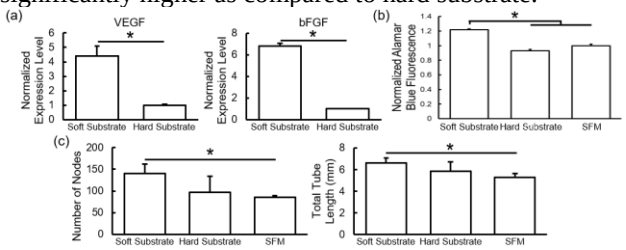

Figure 2. (a) VEGF and bFGF expression of MSCs cultured on soft and hard substrate at transcription level. The effect of secretome collected from MSCs on soft substrate and hard substrate on (b) HUVECs proliferation and (c) HUVECs tube formation, with SFM as control.

Conclusions:

MSCs cultured on soft substrate (i.e. PA hydrogel) successfully promoted the secretion of pro-angiogenic proteins by MSCs, which was then used as supplemental growth factors in promoting the proliferation and tube formation of HUVECs. The expression of pro-angiogenic protein is generally achieved through ROS-NRF2-HIF signaling pathway, and it is the first-of-a kind investigation that systematically studied the effect of substrate stiffness on MSCs pro-angiogenic potential together with the underlying biological mechanism.

References:

[1] STEM CELL TRANSL MED. 2017;6:1445-1451.
[2] CYTOTHERAPY. 2016;18:13-24.