Subcellular Spatial Transcriptional Profiling of Mesenchymal Stem Cells in Hydrogel by Multiplexed RNA Imaging

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Introduction: Mesenchymal stem cells (MSCs) can differentiate into bone, cartilage, and adipose tissue cells. They are good candidates for regenerative therapies for diseases such as cardiovascular degeneration and arthritis. Organoids, spheroid, and aggregates have utilized various synthetic ECM-like 3D network to support stem cell functions. (Cruz-Acuña, R Nat. Protoc 13, 2102–2119 (2018)) We cultured umbilical-cord-derived MSCs (UC-MSCs) and bone-marrow-derived MSCs (BM-MSCs) IN PEG-4MAL hydrogel with GFOGER peptide to mimic collagen-I ECM protein. We then conducted 20-plex RNA fluorescent in-situ hybridization (RNA-FISH) and segmentation staining, and applied machine learning technique to study the subcellular and cellular organization and distribution of mRNA of MSCs to understand the effect of 3D hydrogel environment on MSCs. (Fig. 1a)

Methods: The hydrogel containing 6% 4 arms PEG-MAL was synthesized using PEG-MAL macromer, adhesive peptides, and crosslinkers. PEG-MAL macromers were tethered with thiolated GFOGER peptide at 1:4 ratio in pH 7.8 HEPES by mixing and incubating them for 30 min at 37°C. All components were diluted using PBS++ solution at pH 7.4 and 1% HEPES. The crosslinker suspension, a 1:1 molar ratio mixture of MMP9-degradable VPM peptide and non-degradable DTT and the cells, were suspended together in a pH 6.4 HEPES buffer. Hydrogels were crosslinked by mixing macromers and crosslinker-cell solutions at 1:1 volume ratio and cured at 37°C for 15 min. Culture media was added to each hydrogel assembly and replenished every three days. The cells were fixed after five days with 4% paraformaldehyde for 15 minutes and permeabilized in 70% ethanol at -20°C overnight. The mRNA targets were then labeled using hybridization chain reaction (HCR) protocol and imaged by a fluorescent microscope. (Choi, MTH Development 15 June 2018; 145 (12): dev165753) Images were captured at multiple z-levels. The background-subtracted maximum intensity projection was then generated and analyzed. The fluorescent signals were then removed by recombinant DNase I. (Coskun, AF, Nat Method 13, 657–660 (2016)) The next set of mRNAs were then labeled with the same HCR protocol. Segmentation staining was conducted after imaging all mRNA targets. Concanavalin A (ConA, conjugated with Alexa-488), phalloidin (PhA, conjugated with Alexa-555), and WGA (conjugated with Alexa-647) antibodies are diluted in DPBS buffer. The solution was then added to the cell sample and incubated at room temperature for 45 minutes. Then the sample is washed with 1x PBS for 3 times, 5 minutes each wash. Samples were then imaged.

Result and Discussion: When imaging, single mRNA molecules appears as diffraction-limited dots. By combining the fluorescent image of mRNA and protein targets, we created a multiplexed image for BM-MSCs (Fig. 1b). The pixels of the multiplex image were then clustered using k-means clustering. The clustering result reveals heterogenous lineage phenotype of MSCs in hydrogel. Each cell expresses varying level of different lineage specific genes.

Conclusion: Subcellular and cellular organization of mRNA and protein molecules of MSCs in hydrogel can be studied using multiplexed RNA-FISH and IF staining. The machine learning method can be applied when analyzing the high-dimensional data to reveal subcellular and cellular spatial organization. Such subcellular and cellular spatial profile can be used to understand heterogenous MSC phenotypes in hydrogel environment.

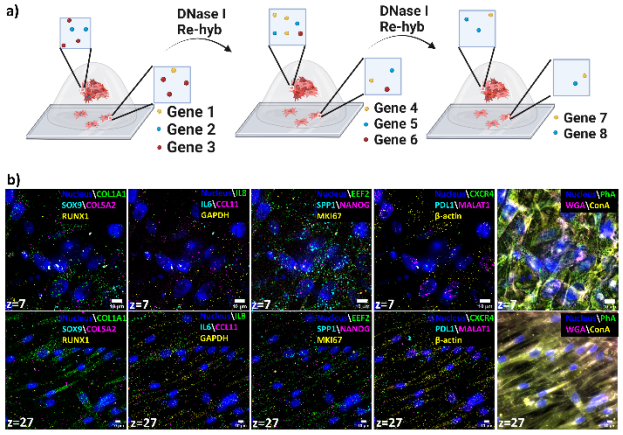

Figure1: a) Illustration of multiplexed RNA-FISH and IF imaging extract subcellular spatial profile of BM-MSCs in 3D hydrogel. b) multiplexed RNA-FISH and IF images of BM-MSCs.

References:

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