Cell-secreted extracellular matrix enhances expression of osteogenic markers in iPSC-MSC spheroids

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DISCLOSURES: None.

INTRODUCTION: Mesenchymal stromal cells (MSCs) have been widely explored for tissue regeneration applications due to their potential to differentiate into both cartilage and bone tissue. However, collection and expansion of bone marrow-derived MSCs remains a critical limitation, particularly in the case of elderly patients where MSCs are sparse and have reduced proliferative and differentiation potential, representing a key limitation for cell-based therapies. As an alternative, MSCs differentiated from induced pluripotent stem cells (iPSC-MSCs) are a promising cell source for regenerative medicine due to their capacity to generate large numbers from undifferentiated iPSCs and potential for autologous sourcing. However, differences in trilineage differentiation and genetic signatures have been reported between human bone marrow-derived MSCs and iPSC-MSCs (iMSCs). In addition to their potent secretome, MSCs also produce key extracellular matrix (ECM) components that are essential for cartilage and bone function, such as collagen, fibronectin, and glycosaminoglycans (GAG). Furthermore, when delivered in the form of cell aggregates (spheroids), the inclusion of a cell-secreted ECM can overcome the initial lack of endogenous ECM for newly formed spheroids and promote lineage-specific differentiation. This endogenous matrix is of vital importance for cell function, as it modulates growth factor production, migration, proliferation, differentiation, adhesion, and apoptosis. Nonetheless, this ECM-loaded approach has not been explored using iMSC spheroids or iMSC-secreted ECM. We hypothesize that the unique composition of iMSC-secreted ECM will increase the expression of relevant osteogenic markers for iMSC spheroids undergoing osteogenic differentiation.

METHODS: iMSC-secreted ECM was produced from human iPSCs in monolayer (P5-P9) culture. ECM was collected by lysing the cells with 0.5% Triton X-100 and 20 mM NH4OH solution, and DNA was removed by incubation with DNase I for 1 h at 37°C. ECM was collected in 0.02N acetic acid and frozen at -20°C until use. We characterized ECM composition with mass spectroscopy and quantification of protein content. One day prior to use, ECM was lyophilized for 24 h and solubilized in growth media. For unloaded spheroids, iMSCs were trypsinized and seeded into nonadhesive molds at 4.35x10^3 cells/mL (15,000 cells per spheroid). Due to their high adhesivity, ECM-loaded spheroids were fabricated individually using a 96deep well plate. To achieve a concentration of 5 µg of ECM per 15,000 cell spheroid, 25 µg/mL of ECM was mixed in a 0.75x10^3 cells/mL suspension, and then 200 µL were pipetted into each well. Plates were then centrifuged for 8 min at 500 Xg and left in static culture conditions with minimum essential alpha medium (α-MEM) for 48 h, after which α-MEM was refreshed with osteogenic differentiation media (OM). Spheroids were cultured statically for an additional 14 d. Spheroids were collected to determine osteogenic gene expression via RT-PCR and calcium content. Other spheroids were fixed for immunocytochemistry. Statistical significance was assessed by one-way or two-way ANOVA when appropriate. Groups with statistically significant differences do not share the same letters.

RESULTS SECTION: Mass spectroscopy revealed that the three main components of iMSC-secreted ECM are perlecan, plectin, and filamin A, which is noticeably different from the collagen-rich ECM secreted by bone marrow-derived MSCs. GAG quantification revealed a higher content in iMSC-secreted ECM (6.6 ± 0.8 µg/mL) compared to ECM from bone marrow-derived MSCs (0.11 ± 0.04 µg/mL). The introduction of iMSC-secreted ECM during spheroid formation increased the expression of osteogenic genes. We observed a significant increase in COL1A1 (collagen type 1) and SPP1 (secreted phosphoprotein 1) expression for ECM-loaded spheroids cultured in OM (p<0.0001) compared to spheroids without ECM in OM. While the expression of RUNX2 (runt-related transcription factor 2) exhibited no significant differences between OM-treated groups (Fig. 1A). Furthermore, calcium deposition per spheroid was significantly higher (3.4 ± 0.1 µg, n=3, p<0.0001) for ECM-loaded spheroids compared to spheroids without ECM (1.6 ± 0.1 µg) after 14 days in OM (Fig. 1B). The expression of osteogenic genes and calcium deposition was significantly lower for spheroids cultured in α-MEM (GM). Furthermore, when cultured in OM without the addition of ECM, calcium deposition and expression of RUNX2 and SPP1 were higher for iMSC spheroids compared to bone marrow-derived MSC spheroids by day 14 (data not shown). Confocal images revealed an increase in F-actin expression for ECM-loaded spheroids compared to those without ECM or spheroids cultured in GM. This indicates changes in cytoskeletal organization that correlate with improved spheroid adhesion and mechanosensing (Fig. 1C). The inclusion of ECM also increased spheroid diameter (n=4 per group) by day 14 (376 ± 27 µm) compared to spheroids without ECM (247 ± 29 µm, p=0.02) or those cultured in GM (242 ± 28 µm, p=0.01) (Fig. 1D).

DISCUSSION: In previously published work, we reported that the introduction of cell-secreted ECM can potentiate osteogenic and chondrogenic potential of MSC spheroids because cells were more responsive to soluble cues in the presence of the cell-secreted ECM. Herein, these data confirm that iMSC-secreted ECM improves osteogenic differentiation in iMSC spheroids compared to unloaded iMSC spheroids. The relatively higher content of perlecan (a basement membrane heparan sulfate proteoglycan) of iMSC-secreted ECM suggests a higher capacity to retain endogenous growth factors, such as BMP-2 or VEGF, that modulate bioactivity of signaling pathways. These encouraging results merit further investigation, as they highlight the importance of developing tissue engineering strategies tailored to the unique characteristics of iPSCs to induce bone regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: These data demonstrate the potential of iMSC-secreted ECM for mineralized tissue formation. These results have the potential to propel the development of tissue engineered constructs that take advantage of the characteristics of iPSCs and their secreted ECM and could serve as the foundation of a novel approach to treating bone defects, especially for elderly patients that have limited availability for autologous bone marrow-derived MSCs.

IMAGES AND TABLES:

Figure 1. Inclusion of iMSC-secreted ECM increases osteogenic potential of iMSC spheroids. GM = spheroids in α-MEM, OM = spheroids in osteogenic media, and ECM OM = iMSC spheroids loaded with iMSC-secreted ECM in OM. (A) ECM OM spheroids exhibit significantly increased COL1A1 gene expression. (B) Calcium deposition and (C) expression of F-actin was higher for ECM OM spheroids. Scale bar = 200 µm. (D) Spheroid diameter remained consistently higher for ECM OM spheroids over 14 days. Data are mean ± SD (n=4).