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Micro-aggregates vs. dispersed cells: what is best for chondrogenic differentiation of BMSCs?

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Introduction
Bone marrow-derived stromal cells (BMSCs) are envisioned as regenerative cells for numerous tissues[1], including cartilage. Success of BMSC-based therapies, however, relies on a number of methodological improvements, among which a better understanding/control of their differentiation pathways. We investigated here if different paracrine signaling and cell-cell contact conditions (micro-aggregates vs dispersed cells) can affect their chondrogenic potential and if different stimulation patterns can modify these effects.

Materials and Methods
Cells and 3D scaffolds: bovine BMSCs (n=3 donors) were encapsulated in alginate beads (1.2%)[1] as dispersed cells and as micro-aggregates at 7 million cells/ml; thus creating different paracrine signaling and cell-cell contact conditions.
Culture conditions: BMSCs were cultured for 21 days at 2%O₂ in high glucose DMEM containing dexamethasone, ITS-1+, BSA, ascorbic-2-phosphate, L-Proline and sodium pyruvate[3]. Medium was supplemented with TGFβ3 (10ng/ml) for 0, 7, and 21 days (Fig. 1).

Data analysis: cell phenotype was characterized by RT-qPCR (type II collagen, sox9, aggrecan); produced matrix by histology (Alcian blue staining) and biochemical assays (glycosaminoglycan (GAG) and DNA content); and cell viability by calcein/propidium iodide staining.

Results
Cell viability (fig.2) and proliferation
• BMSCs stayed viable in all conditions and DNA content increased slightly after 21 days for all dispersed conditions.
• BMSCs formed aggregates after 21 days, with bigger structures under micro-aggregate conditions and 21 days of TGFβ treatment.

Cell phenotype – Chondrogenic differentiation (fig.3)
• All markers increased in time for all groups.
• Higher up-regulation for dispersed cells than for micro-aggregates.
• Higher up-regulation for 7 days of TGFβ treatment.

Matrix production (fig.4)
• GAG/DNA content increased in time for all groups.
• Higher up-regulation for 21 days of treatment and dispersed conditions.
• Matrix deposition increased with days of TGFβ treatment.

Conclusion
• In all conditions, bovine BMSCs differentiated toward the chondrogenic phenotype.
• Cell-cell contact (micro-aggregates) has a negative effect on chondrogenic marker expression but it is not reflected at the matrix level.
• The up-regulation of chondrogenic marker expression was higher for 7 days of TGFβ treatment but the matrix production was higher for 21 days of treatment.
• Overall, chondrogenic differentiation of bovine BMSCs is better for dispersed cells and 21 days of TGFβ treatment.

References