Macromolecular Crowding (MMC) Improves the Microenvironment During Adipogenesis of Human Mesenchymal Stem Cells
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Introduction
Human bone-marrow mesenchymal stem cells (MSCs) reside in a physiological environment crowded by extracellular matrix (ECM) macromolecules and solutes1. In contrast, contemporary culture systems are characterized by cell layers flooded with aqueous medium, resulting in the dilution of signaling molecules and inefficient ECM deposition2. Ex vivo differentiation of MSCs is modulated by defined soluble factors and cell-ECM interactions; both which are affected by macromolecular association kinetics. We hypothesize that the addition of a neutral polymeric cocktail to affect macromolecular crowding will recapitulate a more physiological micro-environment in vitro which could function to enhance the deposition of a lineage-specific ECM; this may in turn augment adipogenic differentiation of MSCs.

Materials and Methods
MMC was effected in vitro by dissolving crowder cocktail in culture media. Adipogenesis was carried out as described3 and assessed via in-situ adherent cytometry of cytoplasmic lipid droplets, flow cytometry (FACS) of lipid droplet expressing cells and lipogenic gene expression. A chaotropic detergent was used to decellularise matrices which were then characterised via immunocytochemistry. Student t-tests were used to determine statistical significance.

Results
MMC drives adipogenic differentiation as observed in increased cytoplasmic lipid accumulation by 25% after 3 weeks induction (Fig.1); FACS also demonstrated a similar trend in the percentage of cells that differentiated, we also observed an increase in fatty acid binding protein (aP2) gene expression (data not shown). MMC enhances the deposition of a lineage-directing matrix as isolated matrices deposited by adipocytes under MMC resulted in twice as much lipid droplets in reseeded MSCs (under adipogenic induction) compared to those seeded on uncrowded matrices (Fig.2).

Discussion and Conclusions
We have shown that macromolecular crowding enhances adipogenesis of MSCs via the enhanced deposition/remodeling of a lineage-specific ECM. This provides a platform that could be used to augment the differentiation of late passage MSCs for tissue engineering. Future studies will focus on the ability of these isolated matrices to drive differentiation in the absence of exogenous induction factors.

References
(3.) Pittenger (1999) Science. 5411, 143 - 147

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Disclosures
Authors have nothing to disclose.