

Fetal Mesenchymal Stem Cell Extracellular Matrix Improves Ex-Vivo Expansion Of Adult Mesenchymal Stem Cells

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Abstract— We investigated the expansion of adult mesenchymal stem cells (aMSC) on extracellular matrix (ECM) derived from fetal human mesenchymal stem cells (fMSC).

I. INTRODUCTION

Large-scale expansion of highly functional aMSCs is technologically challenging as they are of low prevalence in clinical specimens and aMSCs lose self renewal capacity and multipotency during traditional long-term culture and their quality/quantity declines with donor age and disease[1-2]. Thus, identification of culture conditions enabling prolonged expansion and rejuvenation of multipotent aMSCs from an aged donor would have dramatic impact in the field of regenerative medicine. Since previous studies have demonstrated superior proliferation capacity and osteogenic potential of fMSCs[3], we hypothesize that decellularized fMSC ECM will provide a culture platform that promotes aMSC self renewal capacity and multipotency, enabling large-scale expansion of this clinically relevant cell type.

II. METHODS

ECM substrates were generated by culturing cells in 6-well tissue culture wares (TCPS) for 14 days, with addition of ascorbic acid to increase the production of ECM in the final 7-8 days. The cells were decellularized through treatment with detergent-containing NH_4OH solution followed by DNase. Passage 3-4 aMSC were seeded on the various substrates (Passage 3 and 4 fMSC ECM, Passage 3 aMSC ECM, fibronectin and TCPS) and cultured for 10 days. Cells were then detached and counted using a hemocytometer. Images were also acquired and analyzed for cell size distribution. In addition, flow cytometry analysis and differentiation assays were performed to assess the immunophenotype and multipotency of the cells cultured under the various ECM substrates.

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III. RESULTS

Expanded aMSCs were more proliferative when cultured on fMSC-derived ECM in contrast to fibronectin-coating, conditioned media (CM), aMSC-derived ECM and traditional TCPS (Fig 1). Furthermore, these aMSCs were smaller in size, exhibited the biomarker signatures as defined by the International Society for Cellular Therapy and stained positive for osteogenic, adipogenic and chondrogenic expressions, which were indications that they had maintained 'stemness'.

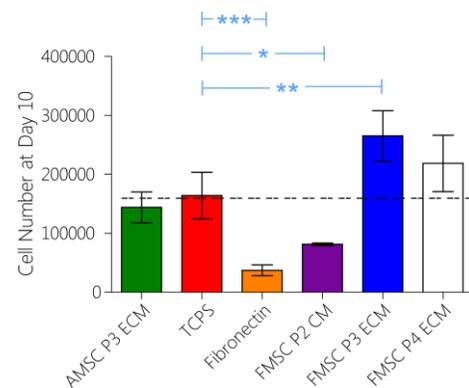


Figure 1. Cell yield of aMSCs cultured under various conditions. Statistical analysis with 1-way ANOVA with Dunnett's Multiple Comparison Test; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

In conclusion, we have demonstrated fMSC ECM as a novel and promising cell culture platform to facilitate the ex vivo expansion of aMSCs.

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REFERENCES

- [1] M.M. Bonab, K. Alimoghaddam, F. Talebian, S.H. Ghaffari, A. Ghavamzadeh and B. Nikbin, "Aging of Mesenchymal Stem Cells In Vitro," *BMC Cell Biology*, vol. 7:14, 2006.
- [2] Y. Lai, Y. Sun, C.M. Skinner, E.L. Son, Z. Lu, R.S. Tuan, R.L. Jilka, J. Ling, and X.D. Chen, "Reconstitution of Marrow-Derived Extracellular Matrix Ex Vivo: A Robust Culture System for Expanding Large-Scale Highly Functional Human Mesenchymal Stem Cells," *Stem Cells and Development*, vol. 19, pp. 1095-1107, 2010.
- [3] Z.Y. Zhang, S.H. Teoh, M.S.K. Chong, J.T. Schantz, N.M. Fisk, M.A. Choolani and J.K.Y. Chan, "Superior Osteogenic Capacity for Bone Tissue Engineering of Fetal Compared with Perinatal and Adult Mesenchymal Stem Cells," *Stem Cells*, vol. 27, pp. 126-137, 2009.