

## Three-Dimensional Culture of Rabbit Bone Marrow Mesenchymal Stem Cells Using Microcarrier Beads in Spin Culture

T. Kamarul<sup>1</sup>, Lily Boo<sup>1</sup>, L. Selvaratnam<sup>2</sup>, Cheh-Chin Tai<sup>1</sup>

<sup>1</sup> Tissue Engineering Group (TEG), Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, MALAYSIA, <sup>2</sup>School of Medicine and Health Sciences, Monash University, Sunway Campus, Selangor, MALAYSIA

**INTRODUCTION:** Bone marrow derived mesenchymal stem cells (MSCs) are attractive candidates for cell-based therapies. However, MSCs expanded in conventional monolayer culture flasks only permits two dimensional expansions thus, resulting in limited and slowly progressing cell proliferation. The objective of this study is to evaluate the feasibility of using commercially available cross linked dextran matrix Cytodex type 1 microcarriers to expand adult MSCs.

**METHODS:** Bone marrows from 3- to 4-month old New Zealand White rabbits were extracted using Ficoll Premium 1.077 gradient centrifugation technique. The isolated cells were pre-characterized (expression of surface marker proteins and differentiation assays) to ensure the isolated cells were MSCs in nature. Once the cells had been characterized, they were expanded in monolayer until P1 before seeding onto microcarriers which is then placed in spin culture systems. At different times over the 14 days culture period, cell growth observations were monitored using an indirect MTT proliferation assay kit, fixed for fixative staining, and processed for scanning electron microscopy. To ensure the cells were still MSCs after expansion, post-characterizations were carried out.

**RESULTS:** In this study, the isolated cells have shown to adhere to tissue culture flasks after 4-5 days of seeding, exhibit fibroblastic-like shape and immunostained positive for MSCs markers CD29 and CD44 (table 1). In the differentiation assay, MSCs have been successfully induced to chondrocytes and osteoblasts respectively. MTT proliferation assay showed Cytodex type I supported the expansion of rabbit MSCs in cell spin culture for up to 14 days. This was consistent with the scanning electron micrographs showing the increase colonization of the cells on the microcarrier beads (figure 1).

Analysis	Result
CD29	Positive
CD44	Positive
CD45	Negative
Chondrogenesis	Positive deposition of cartilage proteoglycans demonstrated by Safranin-O staining
Osteogenesis	Positive deposition of calcium mineralization demonstrated by Alizarin Red S staining

Table 1. Characterization of rabbit MSCs using histology staining (H & E), immunostaining (CD markers) and lineage differentiation assays

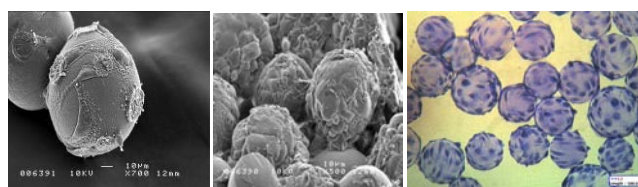


Fig1. Distribution and attachment of MSCs on cytodex type I microcarriers in spin cell culture shown by SEM and light microscopy.

**DISCUSSION & CONCLUSIONS:** We found that the three-dimensional expansion of MSCs on microcarrier provides a simplified mass expansion as compared to conventional two-dimensional cultivation method. In scanning electron micrographs (SEM), they showed excellent cell attachment, spreading and expansion of the cells on Cytodex type I microcarriers. Preliminary analysis from this study has been promising which may pave an alternative way to boost MSCs cell yield for the use in cell-based therapies.

**REFERENCES:** Barry FP & Murphy JM. The International Journal of Biochemistry & Cell Biology. 2004;36(4):568-84. Frondoza C et al. Biomaterials. 1996;17(9):879-88.

**ACKNOWLEDGEMENTS:** This research was funded by National Biotechnology Directorate, Ministry of Science and Technology of Malaysia (MOSTI). (Research grant number: FS116/2008A & FS117/2008A)