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# Perfluorocarbons – potential for successful expansion of bone marrow-derived human mesenchymal stem cells in a liquid/liquid two phase system

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**INTRODUCTION:** The traditional *in vitro* culture systems of adherent cells typically make use of solid surfaces. However, that approach generates monolayers that require the use of harsh enzymatic treatments for cell harvest which could result in the long term damage of cell adhesion proteins[1]. Our solution is using a non-toxic, biocompatible and cost effective liquid/liquid system as an alternative to the complicated, polymer-based systems currently available on the market. A „flexible surface" provided by the interfacial area of two immiscible liquids: a perfluorocarbon liquid (hydrophobic; Fluorinert FC40) and cell culture medium (hydrophilic) is to be used as a cell culture platform. The benefits of using perfluorocarbon liquids to cell culture systems include: easy sterilisation, recoverable and recyclable and scalability[2].

**METHODS:** Bone marrow derived hMSC were seeded at 5,000 cells/cm$^2$ on the interfacial area formed between the two immiscible liquids. The cells were kept in culture for up to 10 days, then harvested by aspirating the interface and replated on TCPS. Cell morphology was assessed by phase contrast and by Live/Dead staining. Cell counts were done with the Nucleocounter NC-3000. Spent medium samples were collected at different time points in culture to assess the metabolic activity of the cells in different conditions and analyzed on the Cedex Bio HT analyser. Cell identity and multipotency were assessed post-harvest by flow cytometry and differentiation studies.

**RESULTS:** hMSCs attached and proliferated on the flexible liquid/liquid interfacial area provided, while exhibiting the typical spindle-like morphology, similarly to traditional TCPS (*Fig. 1a*). Moreover, cells remained viable during culture in the liquid/liquid system (*Fig. 1b*). The cells were expanded at the interface with the option of harvesting as intact cell sheets without the use of proteolytic enzymes by simply aspirating the interface. After 10 days in culture on the flexible interface, harvested hMSCs were successfully sub-cultured on TCPS. In addition, the harvested cells maintained their identity as demonstrated by flow cytometry and multipotency by differentiation and CFU-F efficiency.

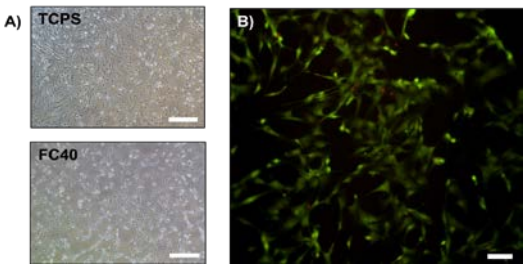


*Fig. 1: A) Phase contrast images of hMSC morphology on TCPS and FC40. B) Live/Dead staining of hMSCs at the interfacial area. Scale bar 500 µm.*

*Table 1. hMSC growth rate on TCPS compared to the FC40/DMEM system*

| | TCPS | FC40/DMEM |
|---|---|---|
| Specific growth rate / h$^{-1}$ | 0.0117 | 0.0061 |

**DISCUSSION & CONCLUSIONS:** Despite the lower growth rates of hMSCs on the flexible interface of the liquid/liquid system compared to TCPS (*Table 1*), the cells remained viable during and post-expansion. Moreover, the cells remained undifferentiated as shown by their expression of CD90, CD73 and CD105 cell surface markers (>95%) and lack of expression of CD34 and HLA-DR markers (<2%). Also, the cells maintained their differentiation potential and ability to form colonies.

**REFERENCES:** [1]Baumann H. and Doyle D. (1979) Effect of trypsin on the cell surface proteins of hepatoma tissue culture cells. *J.Biol. Chem.*, **254(10)**:3935-3946. [2]Lowe K.C. et al. (1998) Perfluorochemicals: their applications and benefits to cell culture. *Tibtech*, **16**:272-277.

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