StemBioSys®

TECHNICAL BULLETIN

Optimized Isolation and Growth of Mesenchymal Stem Cells
The HPME® Advantage

Introduction

Human mesenchymal stem cells (hMSCs) are a promising tool for therapeutic applications in cell-based therapy and regenerative medicine. Although stem cells have emerged as the future of health care, there are still a number of unmet needs, which must be addressed before widespread clinical and therapeutic applications become a reality. One of the major challenges in cell therapy is obtaining sufficient numbers of quality stem cells. Stem cells are relatively rare, in the body, and have a tendency to lose their potency during in vitro expansion.

Surprisingly, the standard for the isolation and growth of hMSCs from mononuclear cells (MNCs) is to extract them from their natural environment and plate them onto tissue culture-treated plastic (TCP). TCP is a foreign environment for the stem cells which induces a rapid response including a shift in gene expression and ultimately to uncontrolled differentiation. It is becoming more broadly accepted that greater attention must be directed to the substrate on which these cells are cultured. This has led to the development of alternative methods for culture including extracted and purified extracellular matrix (ECM) proteins that can be coated or gelled onto the cell culture surface.[1] These substrates still fall well short of mimicking the natural microenvironment from which the cells were extracted.

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StemBioSys has developed an advanced stem cell culture system that replicates the 3-dimensional "home" or microenvironment in which stem cells naturally reside and proliferate.[2] This unique technology enables users to isolate and grow hMSCs from a variety of sources including but not limited to adipose, bone marrow and umbilical cord blood/tissue.

BM-HPME® (Bone marrow-High Performance Micro Environment), is a natural ECM constructed by human bone marrow mesenchymal stem cells (hBM-MSCs). This novel biologic substrate closely mimics the cells natural environment in the body allowing for enhanced isolation and growth of these cells.

Here we directly compare StemBioSys' BM-HPME® with other MSC culture systems including BioCoat™ surfaces, CELLStart™ and MesenCult™, for the isolation and growth of hBM-MSCs.

Materials and Methods

Study Design

Fresh human bone marrow mononuclear cells or previously isolated and frozen mesenchymal stem cells were seeded onto various culture systems at equal seeding density. Cell isolation, proliferation, morphology, and immunophenotyping were monitored.

Bone Marrow from Young Donors for Primary Cell Isolation

Fresh human BM from young donors was purchased from LONZA (Walkersville, MS, USA). The substrates and medias used are described below. Once colonies began to form, media were removed, non-adherent cells washed away gently using PBS, and fresh media added. After the first media change, half-media was changed every 3-4 days. After times indicated, cells were imaged and detached by enzymatic digestion (trypsin or collagenase, depending on the substrate), prior to counting. BM from three unique donors were used for this study.

Bone Marrow Mesenchymal Stem Cells for Proliferation

BM-MSCs previously isolated on TCP by adhesion, and expanded in standard growth media (described below) for 2 passages before freezing in liquid nitrogen were used to assess proliferation. MSCs from three unique donors were used in this study.

Substrate and Media Preparation

BM-HPME® was prepared as previously described.[3] Briefly, BM-MSCs were seeded onto fibronectin-coated 6-well plates. After being induced to secrete abundant matrix protein, the plates were decellularized using a low concentration detergent on the surface of the dish. The remaining decellularized matrix was then washed and then dried for future use. Dried matrix, stored at 4C, is a ready-for-use product and has a shelf-life of up to 12 months.

Mesencult™ (MC) substrate was prepared according to the product insert. Briefly, substrate solution was prepared by adding 133uL of substrate to 39.87mL of PBS. 1mL of substrate solution was added to each well and incubated at room temperature for 2 hours before seeding cells.

CELLstart™ Substrate (CS) was prepared according to instruction in the product insert. Briefly, substrate solution was prepared by making a 1:50 dilution of substrate material in PBS. 750uL of substrate solution was added to each well and incubated at room temperature for 2 hours before seeding cells.

Cells on TCP, BM-HPME®, Corning® BioCoat™ Fibronectin (BC-FN) and BioCoat™ Collagen (BC-C1) were cultured in α-minimum essential medium (MEM) + 2mM L-Glutamine, 1% streptomycin-penicillin, and 15% fetal bovine serum (FBS). Cells on MC and CS were cultured in commercially available media sold in conjunction with the substrates.

Results

Primary cell isolation from fresh bone marrow mononuclear cells

Fresh human bone marrow mononuclear cells (LONZA) were seeded onto TCP, BM-HPME®, and four other commercially available products at $5x10^5$ cells/cm^2. Cells were monitored during 14-day culture. On BM-HPME®, cells were rapidly adherent and proliferation was apparent by day 4. On other substrates, little or no cell adherence was observable 96 hours after cell seeding (Data not shown). Following two weeks in culture, cells were imaged and counted. With the exception of the CELLStart™ substrate, which appeared to isolate mostly macrophages, and the MesenCult™ substrate, which little or no fibroblastic cells adhered, all other substrates isolated cells with a similar, characteristic spindle-like morphology (Fig 1A). In three attempts, we only succeeded in isolating an appreciable number of fibroblastic cells on one occasion from either of these substrates. In contrast, the other four substrates successfully isolated MSCs on each attempt. Cell counts confirmed visual observations (Fig 1B). There is no statistical difference between TCP and the BioCoat™ substrates. Each of these resulted in roughly half as many cells as BM-HPME® after 14 days. The CELLStart™ and MesenCult™ substrate each failed to isolate a substantial number of cells.

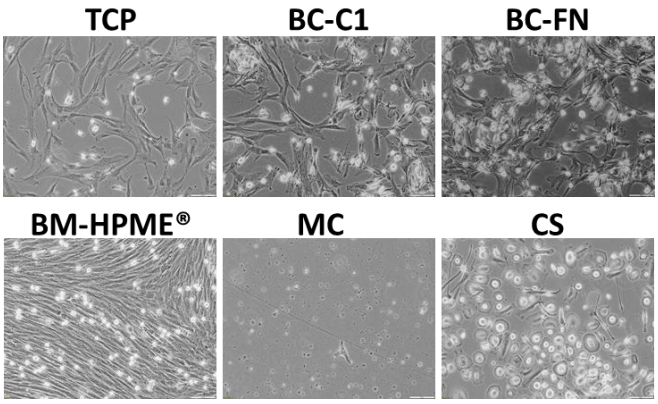

FIGURE 1A. **Isolation of MSCs from bone marrow mononuclear cells**
Brightfield images taken at day 14 demonstrate that MSCs isolated on BM-HPME® appear small and uniform in a densely packed monolayer. Cells on other substrates had substantially fewer cells.

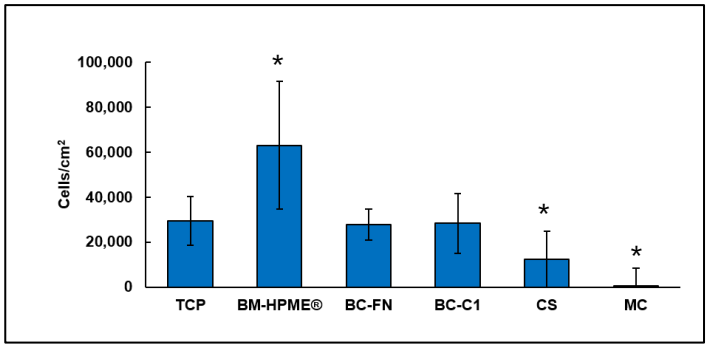

FIGURE 1B. Day 14 Primary Cell Isolation
Cell counts confirmed our visual observations. BM-HPME had significantly more cells than all other substrates ($p<0.05$), whereas CS and MC substrates resulted in fewer cells than TCP following 14 days culture ($p<0.05$). * = $p<0.05$ vs. TCP, t-test. N=3 independent studies.

Proliferation of MSCs and SSEA-4 Expression

BM-MSCs, isolated as previously described, were seeded at 6,000 cells/cm2 and allowed to proliferate. After 4 days, cells were imaged, before being detached for counting followed by immunophenotyping by flow cytometry. MSCs cultured on BM-HPME® rapidly approached confluence and maintained a small, spindle-like morphology relative to TCP or BioCoat™ substrates where a larger, more round morphology was dominant (Fig 2A). Cell counts were multiplied by the percentage of cells expressing Stage Specific Embryonic Antigen-4 (SSEA-4), in order to obtain a better estimate of early stage stem cells present in each culture condition (Fig 2B). Interestingly, BM-HPME® was the only substrate that yielded a significantly greater number of SSEA-4 positive cells, relative to TCP. The MesenCult™ culture condition was the only condition to yield significantly fewer SSEA-4 positive cells than TCP after 4-day culture.

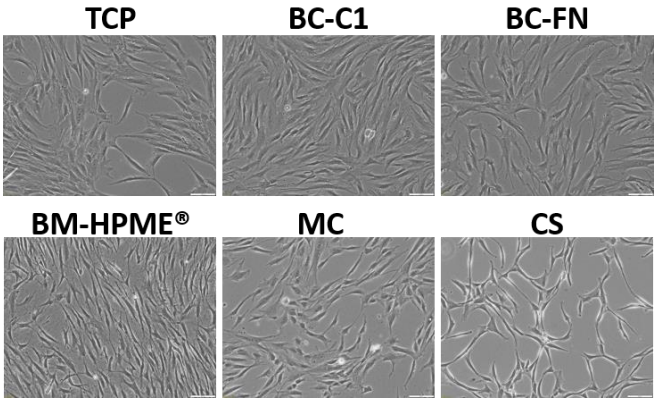

FIGURE 2A. Brightfield images taken at day 4 demonstrate that MSCs expanded on BM-HPME® appear smaller and are more densely packed than cells expanded on other substrates.

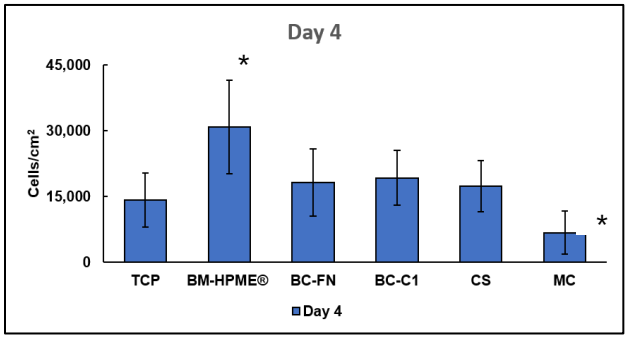

FIGURE 2B. BM-HPME had significantly more cells than other substrates ($p<0.05$), whereas the MC substrate resulted in fewer cells than all other substrates following 4 days of culture ($p<0.05$). * = $p<0.05$ vs. TCP, t-test. N=3 independent studies.

Discussion

It is now widely accepted, that appropriate stem cell-matrix interactions are necessary for maintaining cell cultures and for obtaining biologically relevant results from in vitro assays.[4] Moreover, in order to develop clinical applications of stem cells, it is important to be able to expand large numbers of stem cells without loss of potency. Still, however, it is unclear what aspects of the extracellular matrix are most critical for determining cell fate. Many products take a reductionist approach and attempt to improve cell culture by coating a plastic substrate in a solution of a single or just a handful of matrix proteins oriented randomly. This approach fails to recognize the complexity of the native niche, and the potential for complex interaction of matrix components and properties. While some substrates may have the potential to briefly increase cell number, maintaining MSC potency is equally if not more important. Currently our understanding of cell matrix interactions does not permit the use of only a few components to recreate the impact of the native niche.

StemBioSys' BM-HPME® differentiates itself from other matrix products by embracing the complexity of the native niche. BM-HPME® is a stem cell-derived product with over 100 unique proteins preserved in their original architecture. This approach results in a substrate that enables more efficient isolation of stem cells from primary cells, and expansion of stem cells without loss of potency.

In vivo, stem cells are able to divide and differentiate to maintain tissues over a period of decades. In vitro, however, it is often impossible to maintain stem cell potency for short periods. As the fields of stem cell biology and regenerative medicine move toward the development of more clinical applications, it is critical that cell culture techniques evolve in order to produce higher quantities of high quality MSCs. This may be accomplished by better mimicking conditions of the native stem cell microenvironment, that enable cells to maintain potency for extended periods. StemBioSys' BM-HPME® represents a one-of-a-kind culture substrate that recaptures critical aspects of the complex stem cell niche to enable better cell culture.

References

1. Shakouri-Motlagh, O'Connor, Brennecke. Native and solubilized decellularized extracellular matrix: a critical assessment of their potential for improving the expansion of mesenchymal stem cells. Acta Biomaterialia. 2017.
2. Lai Y, Sun Y, Skinner C, et al. Reconstitution of marrow-derived extracellular matrix ex vivo: a robust culture system for expanding large-scale highly functional human mesenchymal stem cells. Stem Cells Dev. 2010.
3. Chen X. Extracellular matrix provides an optimal niche for the maintenance and propagation of mesenchymal stem cells. Birth Defects Research Part C: Embryo Today: Reviews. 2010.
4. Marinkovic M, Block TJ, Rakian R, et al. One size does not fit all: developing a cell-specific niche for in vitro study of cell behavior. Matrix biology : Journal of the International Society for Matrix Biology. 2016.

StemBioSys BM-HPME® Products

Vendor Part Number	Description
BM-HPME-6WP	BM-HPME, 6-well plate
BM-HPME-T-75	BM-HPME, 75cm2, T-75
BM-HPME-T-150	BM-HPME, 150cm2, T-150
BM-HPME-100mm	BM-HPME-100mm

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