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A Unique Defined Pre-Coating - Free Culture Platform for Isolation and Expansion of hMSC Towards Clinical Applications

Mira Genser-Nir, Sharon Daniliuc, Marina Teverovsky, Yuliya-Yael Miropolski, Maria Sharovetsky, Roni Hazan Brill, David Fiorentini

Biological Industries (BI), Beit Haemek, Israel

Abstract

Human mesenchymal stem cells (hMSC) hold great promise as tools in cell therapy. Since hMSCs are rare in adult tissues, the isolated cells must be expanded in-vitro to generate sufficient cells number for clinical use.

The use of serum-free (SF), xeno-free (XF) culture system is an advantage in order to minimize the health risk of using xenogeneic compounds, and to limit the immunological reactions in-vivo. Under SF, XF culture condition, a coating step is usually required to enable cells attachment, spreading and proliferation in-vitro.

The coating procedure is an obstacle step for scale up towards cells therapy. Thus, having an optimal coating- free culture platform may provide efficient, user-friendly and economical hMSC manufacturing process.

In the present study, different combinations of treated plastic ware (uncoated) and SF, XF media were evaluated.

Results show that MSC NutriStem® XF (BI) together with Corning® CellBIND® (uncoated culture ware) is a superior platform for the expansion and proliferation of hMSC from a variety of sources.

Materials and Methods

Cells
hMSC-AT (ATCC, fat tissue), BM, WJ and DP (Lonza) were used in this study.

Initial isolation of hMSC
hMSC-AT were isolated from Healthy donor's fat tissue by enzymatic digestion followed by centrifugal separation to isolate the stromal | vascular cells . Pellet was re-suspended with MSC NutriStem® XF supplemented with 2.5% human AB serum and cultured on CellBIND® (without pre-coating procedure) and on pre-coated TC dish (using 05-752-1; BI). Human AB serum was added only at passage 0 (for the initial isolation step).

XF, SF Culture system
hMSC were cultured in MSC NutriStem® XF on CellBIND® or pre-coated TC dish (using 05-752-1; BI). Cells were seeded at concentration of 4000-5000 viable cells/cm² and harvested using Recombinant Trypsin Solution (03-078-1; BI).

Medium performance evaluation
Medium performance was evaluated by viable cell count and PDL calculation (indication for proliferation rate), cell morphology, multilineage differentiation potential into adipocytes, osteocytes, and chondrocytes, self-renewal potential and cell's immunophenotype.

Differentiation
hMSC expanded for 2-3 passages in MSC NutriStem® XF on CellBIND® were tested for multilineage differentiation potential (into adipocytes, osteocytes, and chondrocytes) using MSCgo™ differentiation media (BI). The Adipogenesis and Osteogenesis assays were done on CellBIND® plate. The Chondrogenesis assay was done in 96w/p U bottom ULA plates for micro-mass culture. Followed by differentiation, cells were fixed and stained with Oil Red O, Alizarin Red, and Alcian Blue, respectively.

CFU-F Assay
hMSC expanded for 2-9 passages in MSC NutriStem® XF on CellBIND® were tested for self renewal potential. For CFU-F assay hMSC were seeded at low density (40 cells/cm²) in MSC NutriStem® XF on TC pre-coated dish (05-752-1, BI) and cultured for 14-18 days followed by staining with 0.5% Crystal violet.

Flow Cytometry
hMSC were cultured for 3 passages in MSC NutriStem® XF on CellBIND® followed by MSC identification by flow cytometry using positive and negative surface markers (eBioscience CD90, CD105, CD73, CD34, CD45, diluted 1:500).

Abbreviations

ACF	Animal Components Free
ARS	Alizarin Red S
CFU-F	Colony Forming Units- Fibroblasts
PDL	Population Doubling
hMSC	Human Mesenchymal Stem Cells
hMSC-AT	Adipose Tissue derived hMSC
hMSC-BM	Bone Marrow derived hMSC
hMSC-WJ	Wharton's Jelly (Cord Tissue) derived hMSC
hMSC-DP	Dental Pulp derived hMSC
hPL	Human Platelet Lysate
SF	Serum Free
TC	Tissue Culture
XF	Xeno Free
ULA	Ultra Low Adherent

Results

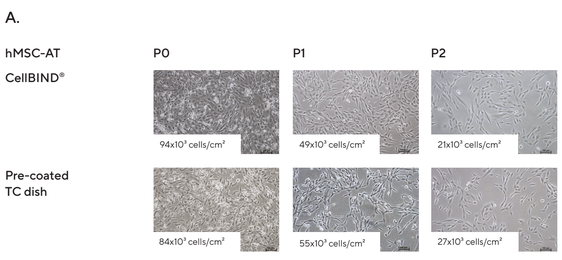
I. Isolation

Figure 1:
Isolation of hMSC under XF culture condition w/o pre-coating procedure is applicable using MSC NutriStem® XF and Corning® CellBIND® Surface

Human adipose tissue derived MSC were Isolated utilizing CellBIND® uncoated culture dish in comparison to control pre-coated TC dish (MSC Attachment solution, BI). The isolated cells were seeded in MSC NutriStem® XF supplemented with 2.5% human AB serum (P0). Further passages were done under XF, SF culture condition using each culture dish, followed by cells evaluation.

A. Representative images (x100) of hMSC-AT taken at day 3 post isolation (P0) and at day 2 of further 2 passages.
B. Average PDL of hMSC-AT isolated and cultured for 3 passages using CellBIND® and pre-coated TC dish.
C. Immunophenotyping results of hMSC-AT at passage 2 using flow cytometry analysis.
D. Differentiation results of hMSC-AT cultured on CellBIND® uncoated culture ware. Adipogenesis -16 days assay (Oil Red O, x400), Osteogenesis -16 days assay (2% ARS, x100) and Chondrogenesis-23 days assay (Alvian Blue, x40).

Successful isolation of hMSC-AT with high yield of isolated cells that maintains classical profile of MSC markers and tri-lineage differentiation potential was achieved utilizing Corning® CellBIND® Surface (w/o pre-coating procedure).



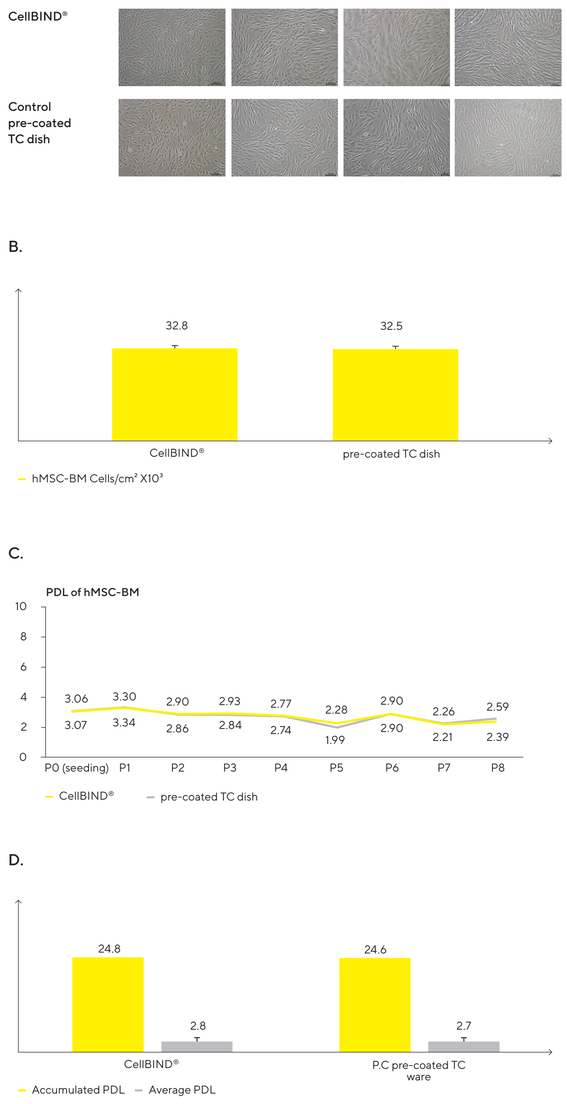
II. Expansion

Figure 2:
Long term proliferation

Long term culturing of hMSC under XF culture condition w/o pre-coating procedure using MSC NutriStem® XF and Corning® CellBIND® Surface.
hMSC-BM were cultured for 8 passages in MSC NutriStem® XF on CellBIND® or pre-coated TC dish (05-752-1; BI). During 8 passages, at each passage the cells were harvested, counted and equally reseeded in each culture dish.

A. Representative Images (x100) of hMSC-BM during the passages.
B. Average of hMSC-BM proliferation (cells/cm² x10³).
C. Population doubling results in each passage.
D. Average of population doubling and accumulated population doubling results after 8P.

MSC NutriStem® XF in combination with CellBIND® enables long term culturing of hMSC-BM under XF culture condition w/o the need of pre-coating step with similar morphology and proliferation as on pre-coated TC dish.



III. Suitability

Figure 3:
Suitability for various types of hMSC

Culture of hMSC from various sources under XF culture condition w/o pre-coating procedure using MSC NutriStem® XF and Corning® CellBIND® Surface.
hMSC derived from a variety of sources: AT, BM, WJ and DP were cultured for 2 passages in MSC NutriStem® XF on CellBIND® or pre-coated TC dish (05-752-1; BI).

A. Representative Images (x100) of various types of hMSC at day 3 post passage.
B. Average of population doubling of hMSC during 2 passages.

MSC NutriStem® XF in combination with CellBIND® enable the culturing of hMSC from different sources under XF culture condition w/o the need of pre-coating step.

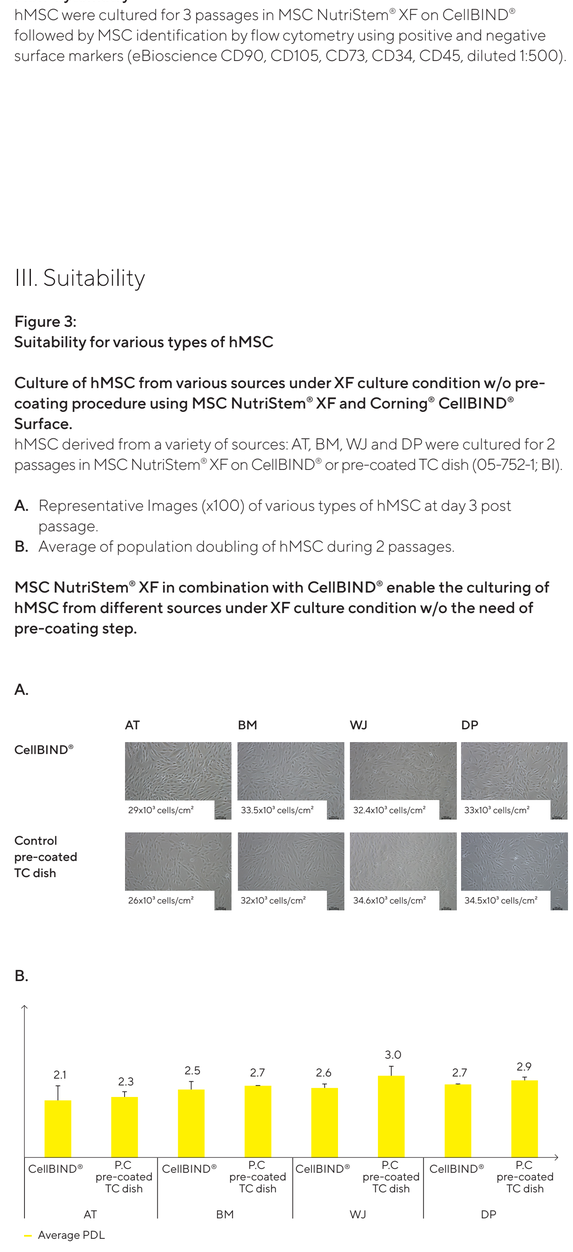
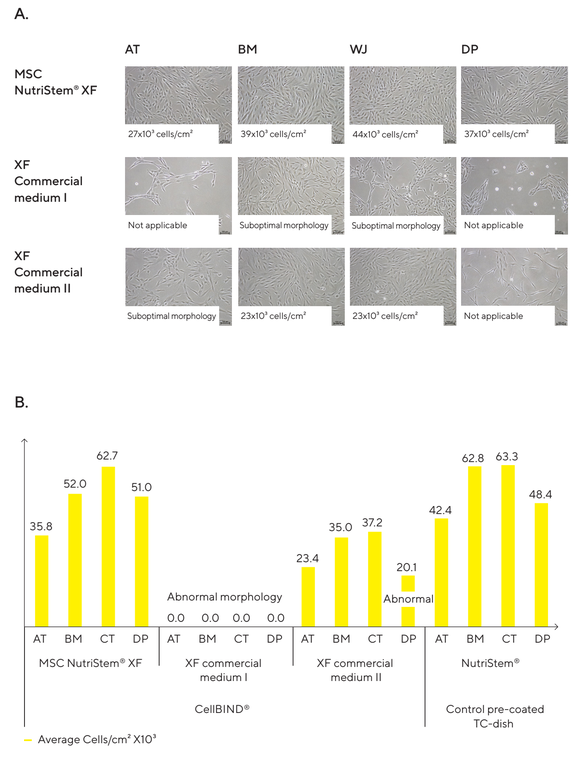


Figure 4:
The SF, XF hMSC culture system composed of MSC NutriStem® XF and Corning® CellBIND® Surface is distinctive

Culturing of hMSC w/o pre-coating procedure on Corning® CellBIND® Surface using MSC NutriStem® XF compared to other commercial SF, XF culture media.
hMSC derived from a variety of sources: AT, BM, WJ and DP were cultured for 2 passages in different SF,XF culture media on CellBIND® Surface. The diffidences between the tested XF, SF media were more significant post passage.

A. Representative Images (x100) of various types of hMSC at day 3 post passage in each media.
B. Average proliferation results of hMSC during 2 passages in different XF culture media on CellBIND® and TC pre-coated dish.

Results show that only MSC NutriStem® XF in combination with CellBIND® enables the culturing of hMSC from different sources under XF culture condition w/o the need of pre-coating step.



IV. hMSC Characterizations

Figure 5:
Immunophenotyping of hMSC

Immunophenotyping results of hMSC-BM cultured for 9 passages in MSC NutriStem® XF on CellBIND® or pre-coated TC dish (05-752-1; BI).

A. Flow cytometry data
B. Summary of marker expression.

hMSC cultured in MSC NutriStem® XF medium on CellBIND® w/o pre-coating procedure maintain classical profile of MSC markers with lower percentage of hematopoietic cells contaminations.

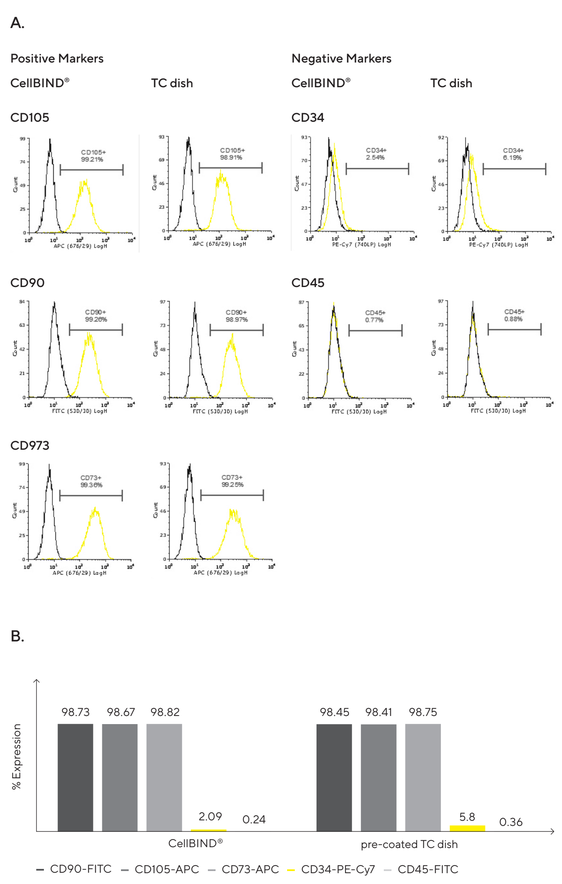


Figure 6:
Trilineage differentiation potential

hMSC-AT expanded in MSC NutriStem® XF medium on CellBIND® for 3 passages followed by differentiation assays using MSCgo™ differentiation media (BI). Representative images of stained a dipocytes (Oil Red O, x400) after 16 days assay, stained osteocytes (2% Alizarin Red, x100) and stained chondrocytes (Alcian Blue, x40) after 23 days assay.

hMSC cultured in MSC NutriStem® XF w/o precoating procedure on CellBIND® Surface maintain their multilineage differentiation potential.

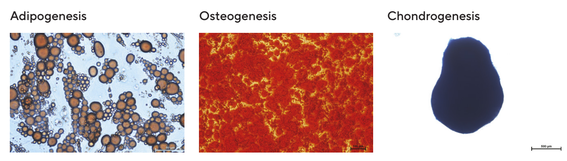
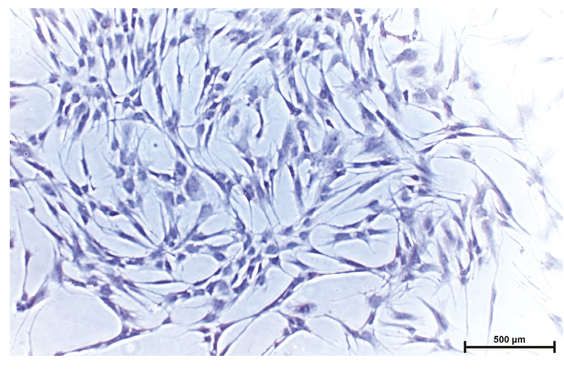


Figure 7:
Self renewal potential

hMSC-BM expanded in MSC NutriStem® XF medium on CellBIND® Surface prior to 18 days of CFU-f assay. Representative image of mature colony stained with 0.5% Crystal violet (x40).

hMSC cultured in MSC NutriStem® XF medium on CellBIND® Surface w/o pre-coating procedure maintain their self-renewal potential.



Summary

- Isolation of hMSC-AT under XF culture condition without a coating procedure is applicable using MSC NutriStem® XF medium with the addition of 2.5% human AB serum and Corning® CellBIND® Surface.
- The supplementation of 2.5% human AB serum is required only for the initial step of isolation.
- Further passages are applicable under fully SF, XF culture conditions and w/o the need of precoating step.
- MSC NutriStem® XF together with Corning® CellBIND® Surface (uncoated culture dish) support culturing of hMSC form different sources (e.g. AT, BM ,CT,DP) while maintaining typical hMSC characteristics (Fibroblast -like morphology, surface markers phenotype, multi-lineage differentiation and self-renewal potential).
- Corning® CellBIND® Surface is superior with MSC NutriStem® XF in comparison to other commercial SF, XF media.
- To conclude: MSC NutriStem® XF (BI) together with Corning® CellBIND® Surface (uncoated culture dish) enable superior platform for culturing of hMSC from a variety of sources without the need of pre-coating step.