A UNIQUE DEFINED PRE-COATING - FREE CULTURE PLATFORM FOR ISOLATION AND EXPANSION OF HMSC TOWARDS CLINICAL APPLICATIONS

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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/cm^2 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/cm^2) 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).

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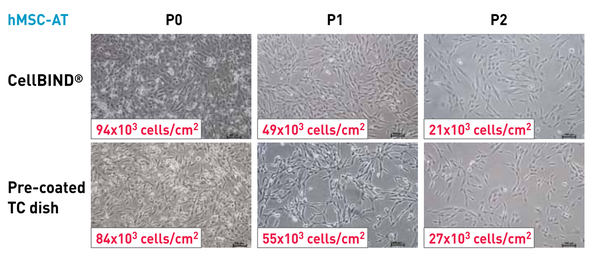

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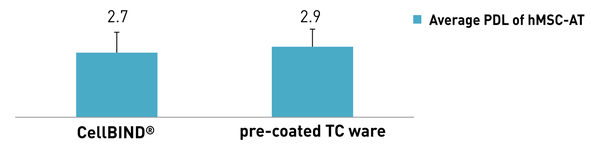

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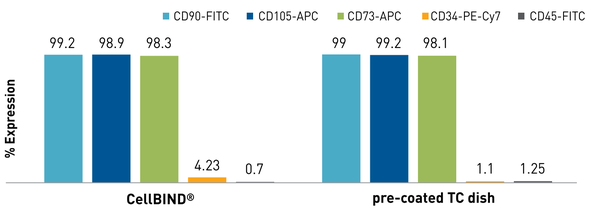

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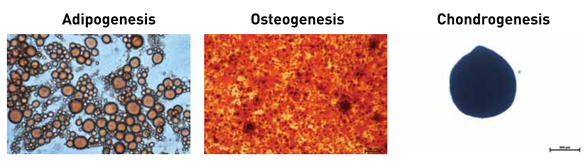


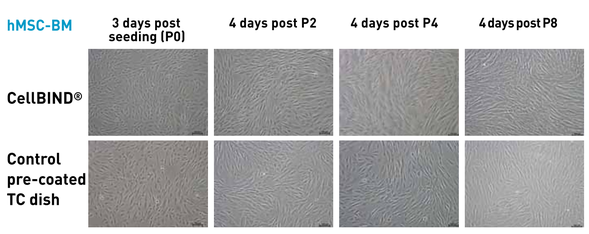
II. Expansion

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/ cm^2 x10^3).
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.

A.



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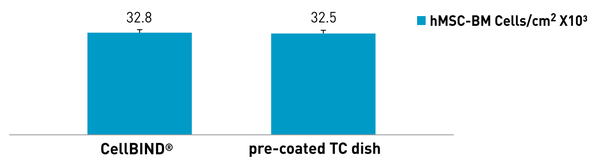

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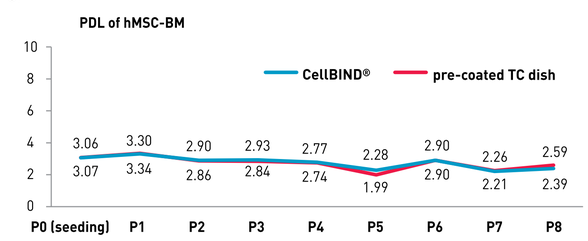

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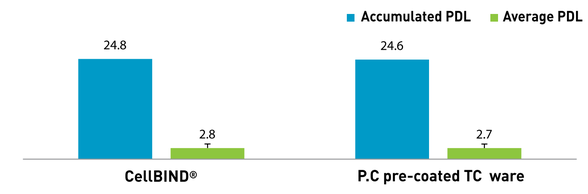

III. Suitability

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.

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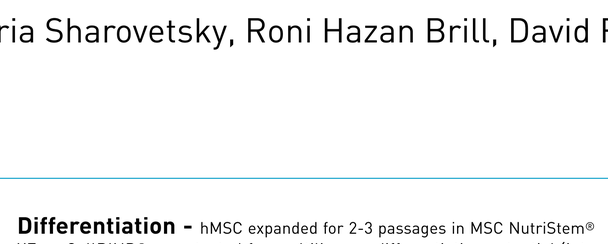

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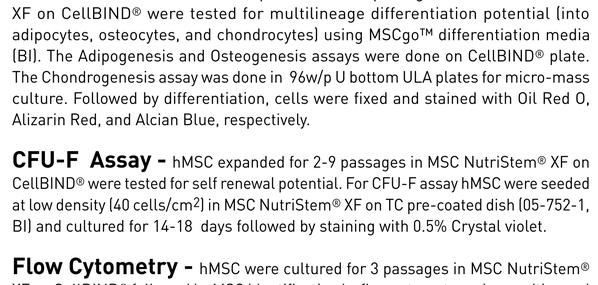

Figure 4:
The SF, XF hMSC culture system composed of MSC NutriStem® XF and Corning® CellBIND® Surface is distinctive

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.

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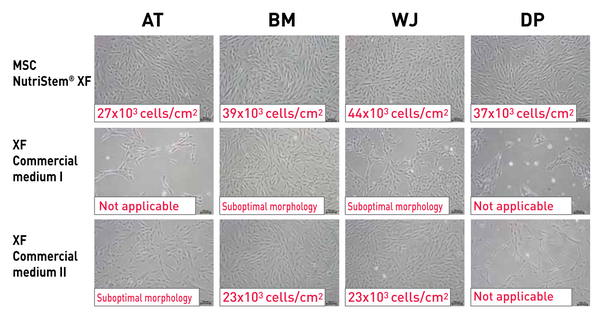

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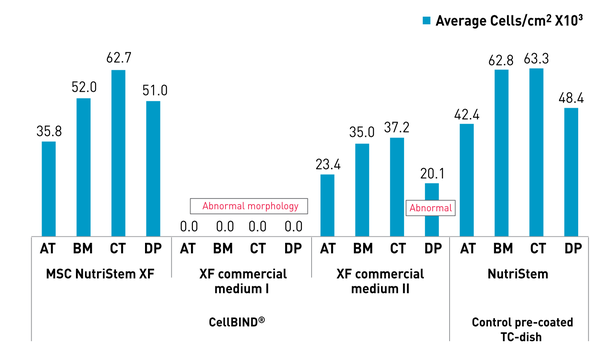


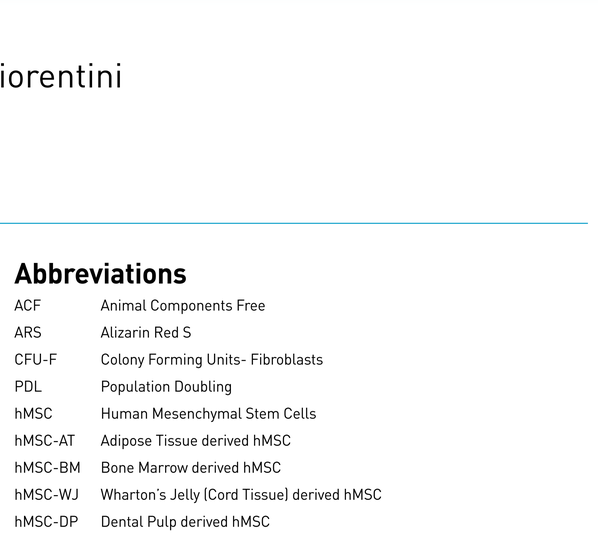
IV. hMSC characterizations

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.

A.



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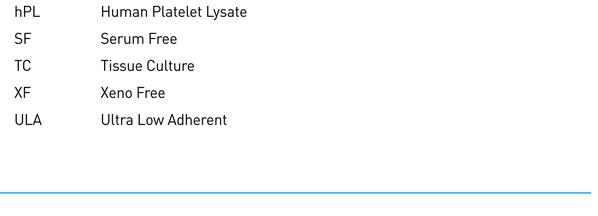


Figure 6:
Trilineage differentiation potential

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.

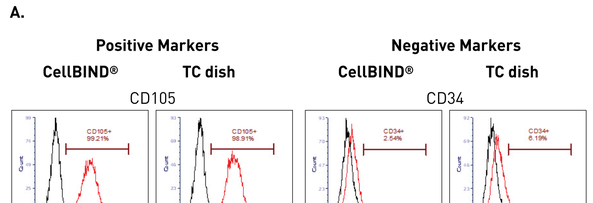
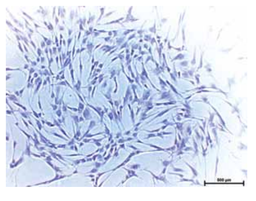


Figure 7:
Self renewal potential

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.