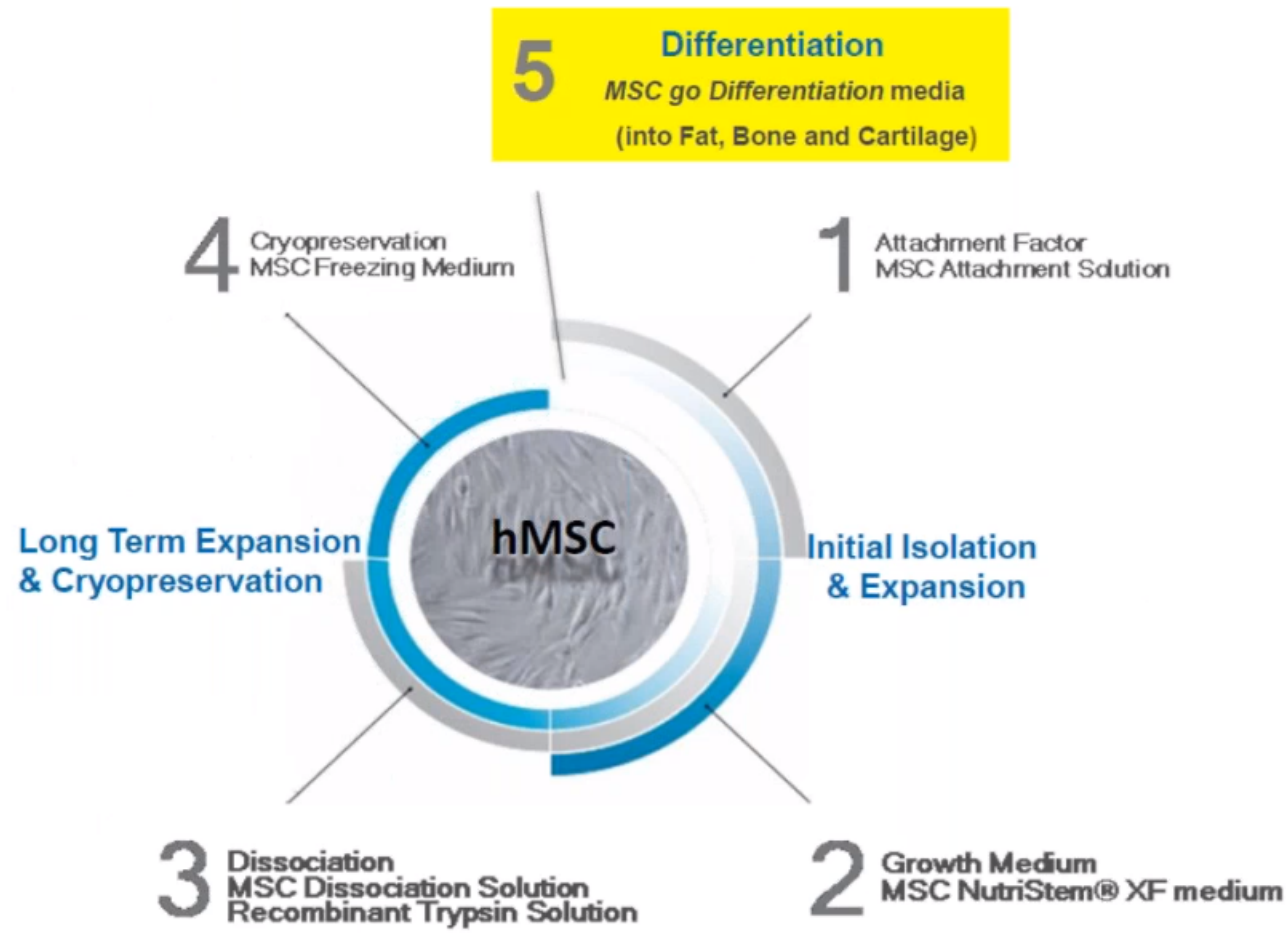


Complete SF, XF platform for hMSCs



MSC NutriStem Xf

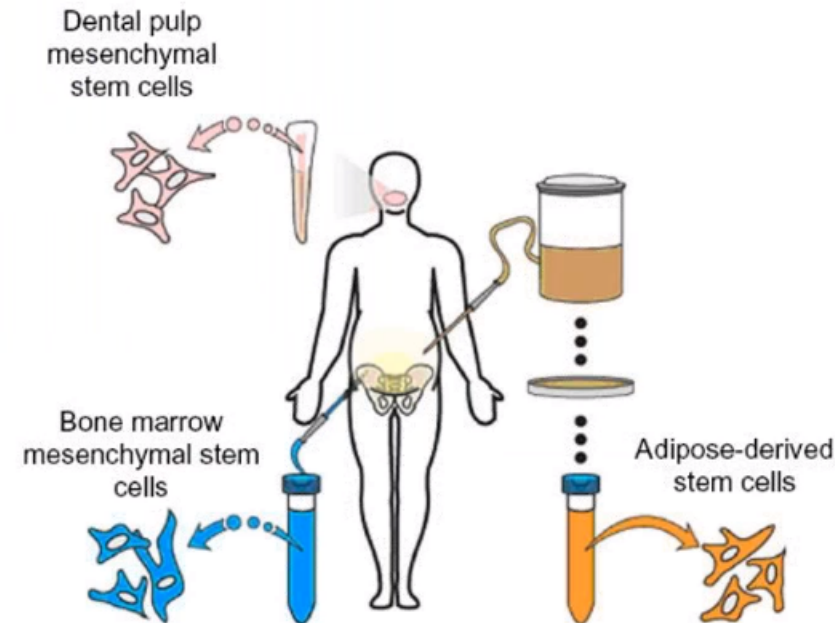
A serum-free (SF), xeno-free (XF) medium specially developed for the isolation and expansion of hMSCs

Product Description	Storage	Cat. No.	Size
MSC NutriStem® XF Basal Medium	2-8°C	05-200-1A	500 ml
MSC NutriStem® XF Supplement Mix	-20°C	05-201-1U	3 ml
MSC NutriStem® XF Basal Medium	2-8°C	05-200-1B	100 ml
MSC NutriStem® XF Supplement Mix	-20°C	05-201-1-06	0.6 ml

Supplement Mix + Basal Medium = complete ready-to-use medium (2-8°C) – up to 30 days

Human Mesenchymal Stromal Cells – Isolation

MSCs from bone marrow, peripheral blood and synovial fluid can be isolated by using Ficoll density gradient method with small modifications and seeded into culture plates. MSCs from various tissue sources (adipose, dental, endometrium, placenta, Wharton's Jelly) can be isolated after digestion with collagenase and then cultured at varying densities. MSCs will keep the undifferentiated character for 5 to 6 passages; while commercial line are well for up to 10 passages



Successful Isolation of AD-hMSC using MSC NutriStem® XF



- Fresh adipose tissue in PBS
- Wash 3 times in PBS
- Treating with Collagenase type IV for up to 120min at 37°C

Tissue digestion



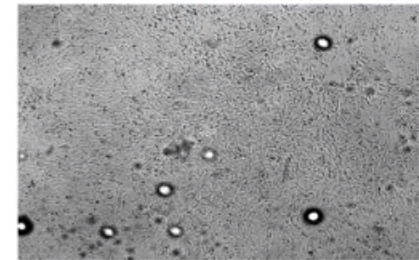
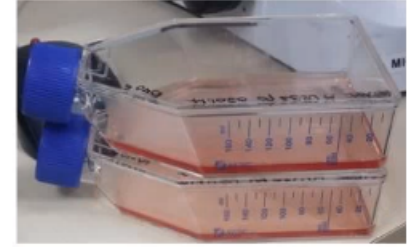
MSC isolation and enrichment

- Neutralize with medium
- Centrifuge 1200rpm for 10 min and wash with PBS
- Add RBC lysis solution
- Wash with PBS



MSC seeding

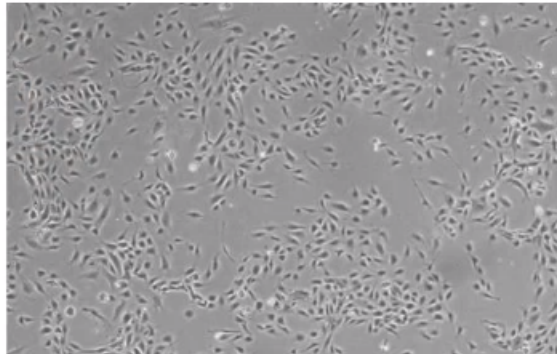
- Suspend pellet in complete media
- Counting cells with Trypan Blue
- Seed culture on plates coated with attachment solution



hMSC-AT
4 days post initial isolation (P0)

Successful Isolation of hMSC using MSC NutriStem[®] XF

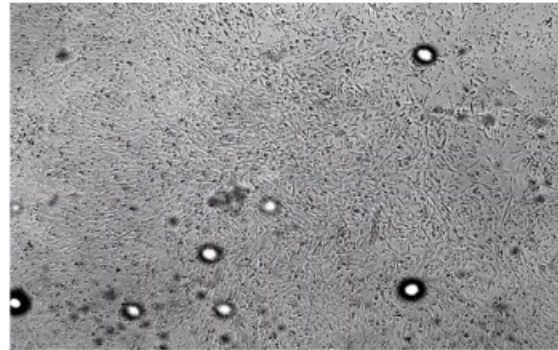
hMSC-WJ



6 days post initial isolation (P0)

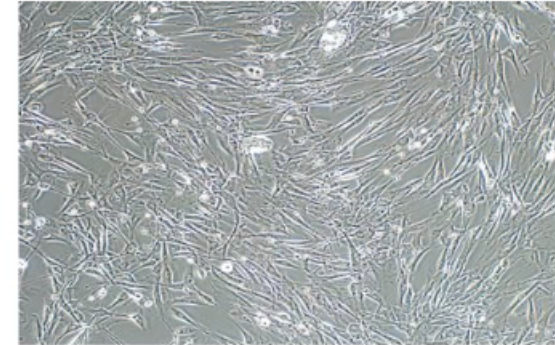
+ supplementation of 2% human AB serum•
on pre-coated plates (MSC Attachment Solution)•

hMSC-AT



4 days post initial isolation (P0)

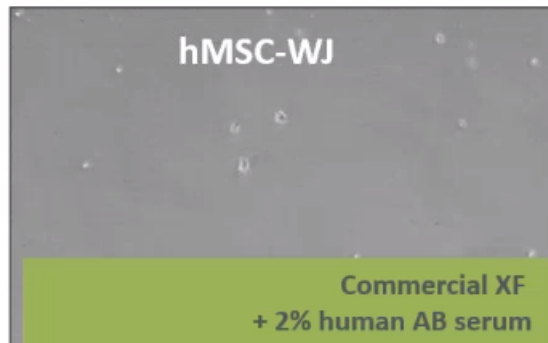
hMSC-PL



11 days post initial isolation (P0)

w/o human AB serum•
on pre-coated plates•
(MSC Attachment Solution)

hMSC-WJ



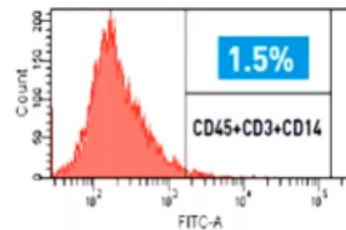
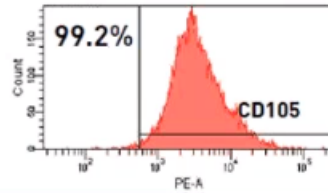
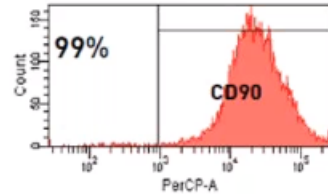
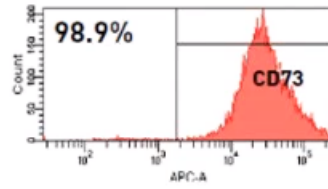
Commercial XF
+ 2% human AB serum

Isolation of hMSC from various tissues is achievable
under XF (SF?) culture system using MSC
NutriStem[®] XF

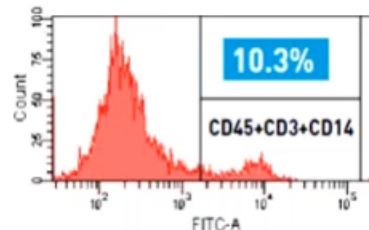
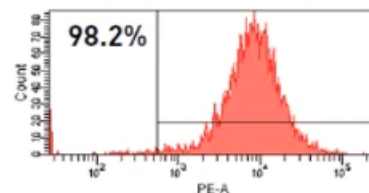
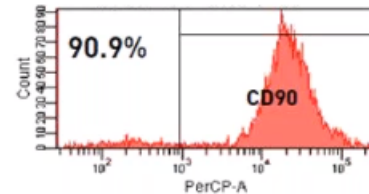
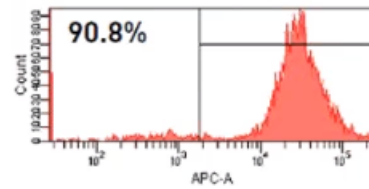
MSC NutriStem XF, Isolation: Profile Markers

hMSC-BM

MSC NutriStem® XF (SF)



Serum containing medium



11 days post initial isolation (P0). •
Immunophenotyping using FACS analysis (P0) •
MSC NutriStem® XF (SF) Vs. •
FBS containing medium

Higher expression of
MSC markers

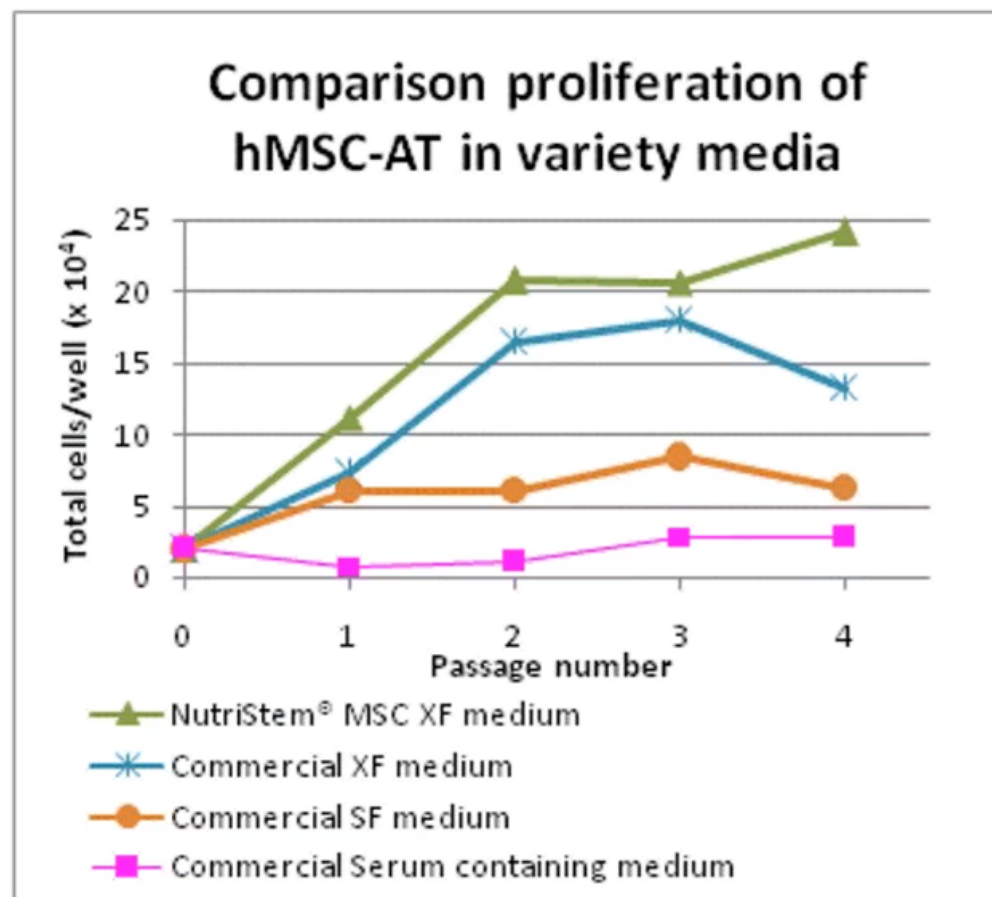
Lower contamination of
hematopoietic cells

Higher level of purity hMSC is observed
utilizing MSC NutriStem® XF Under complete SF, XF culture condition

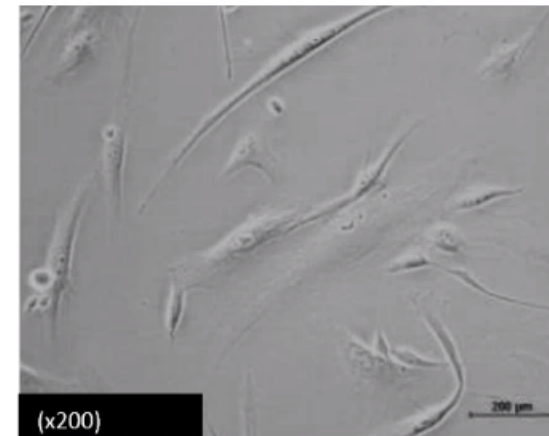
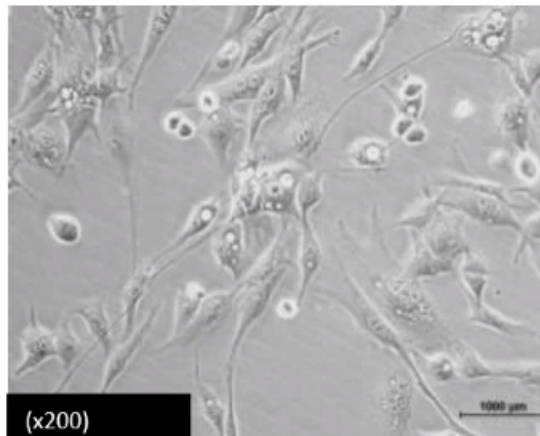
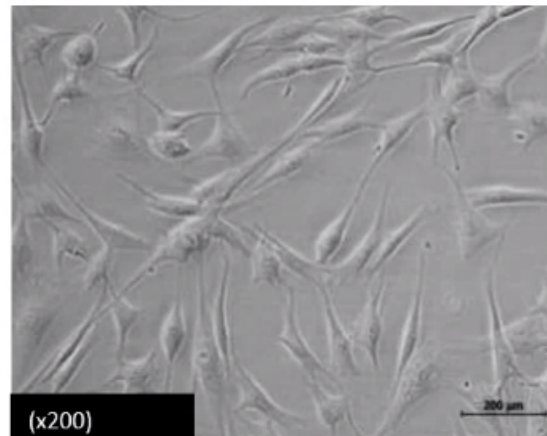
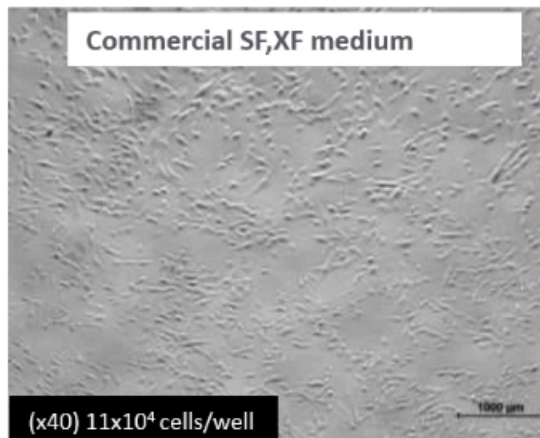
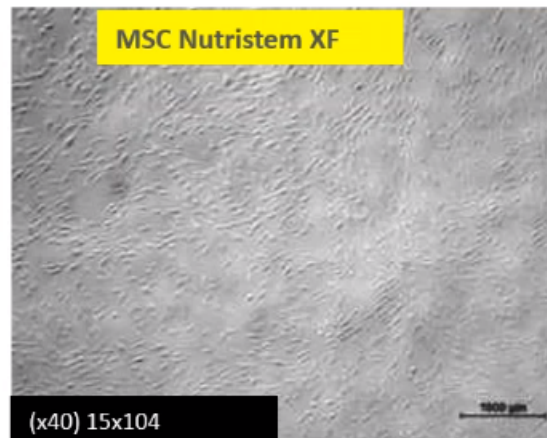
Human Mesenchymal Stromal Cells – Expansion

hMSC-AT cultured in MSC NutriStem® XF exhibit superior proliferation in comparison to serum-containing medium and commercially available SF and XF media.

- Total cell number per well (12 well-plate)
- Seeding density: 2×10^4 cells/well. Passage frequency of 3 days.
- The culture plates were pre-coated with MSC Attachment Solution (BI)



MSC NutriStem XF, Expansion: normal morphology

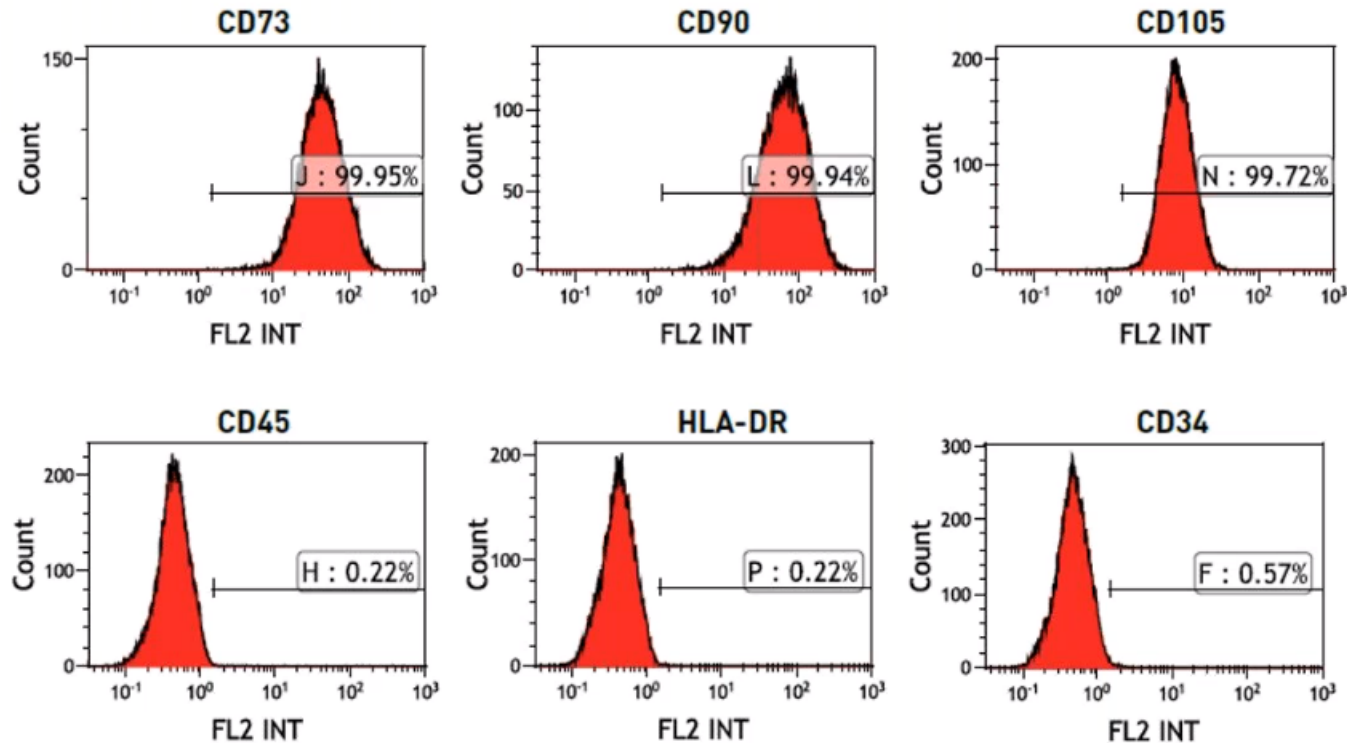


hMSC-AT expansion in MSC NutriStem® XF & commercially SF available media

Typical fibroblast-like cells morphology when using MSC NutriStem® XF

MSC NutriStem XF, Expansion: Profile Markers

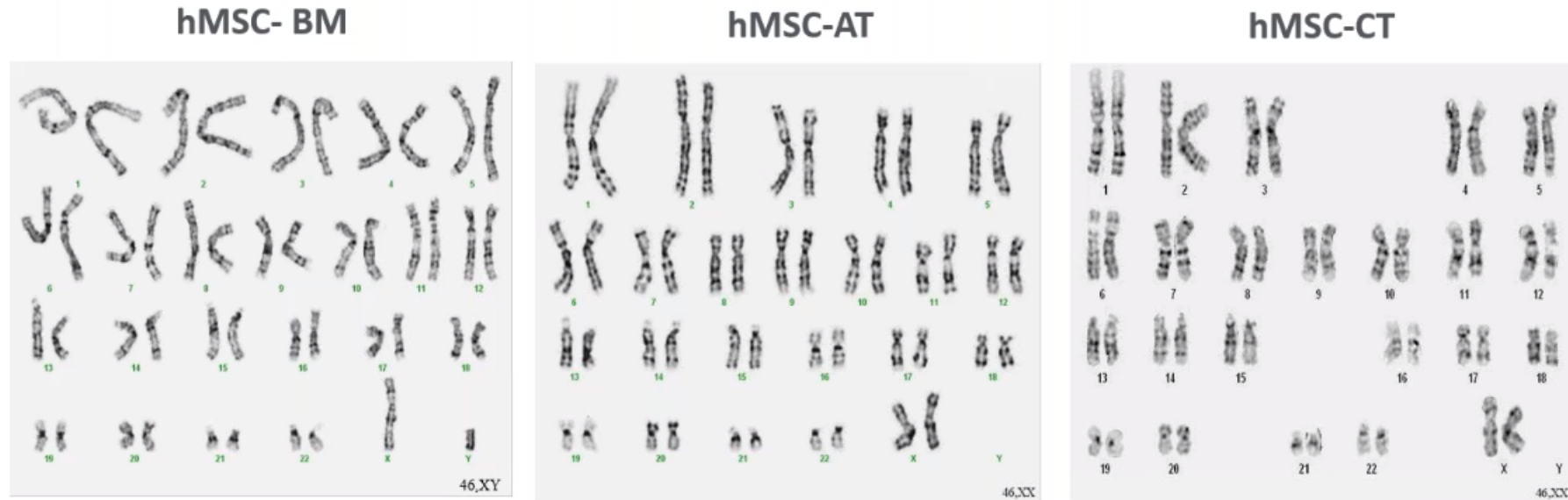
Immunophenotyping results of **hMSC-AT** at passage 2 using FACS analysis



hMSC maintains a classical profile of MSC markers using MSC NutriStem® XF, MSC Attachment Solution

MSC NutriStem XF, Expansion: Karyotypic Stability

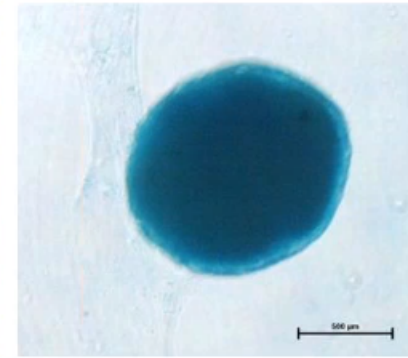
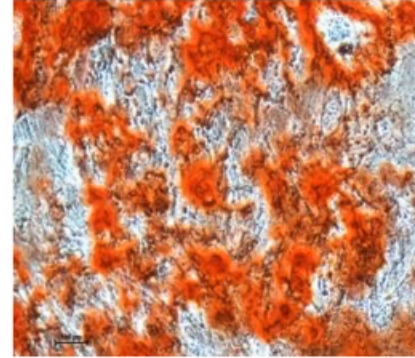
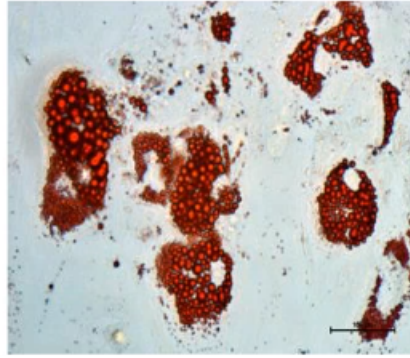
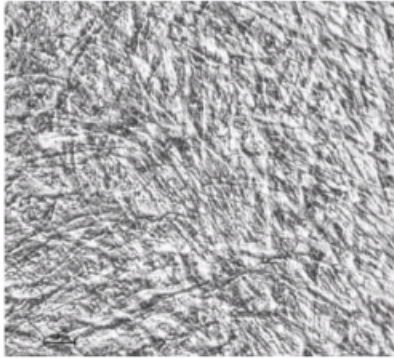
G-banding karyotype analysis of hMSC-BM and hMSC-AT cultured for 4-6 passages in MSC NutriStem® XF.



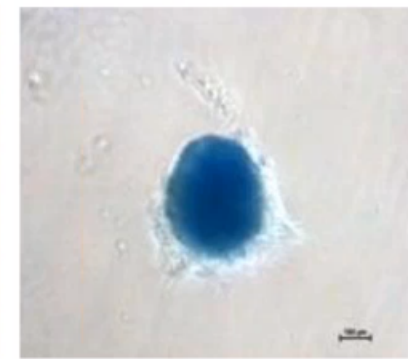
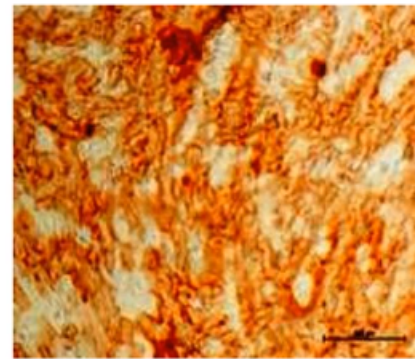
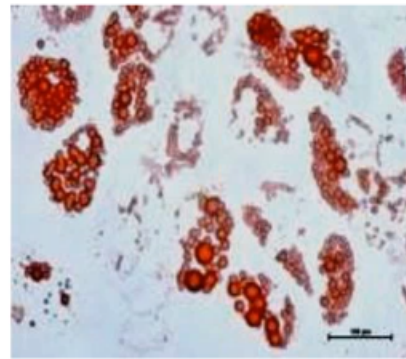
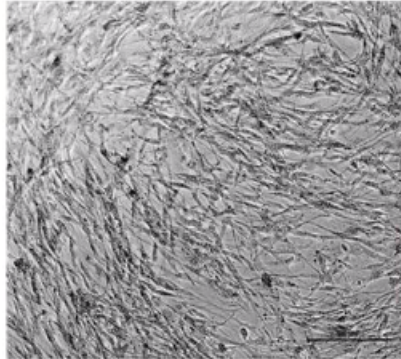
hMSC cultured in MSC NutriStem® XF medium maintain genomic stability

MSC NutriStem XF, Expansion: Tri-lineage differentiation

hMSC -BM



hMSC -AT



Control

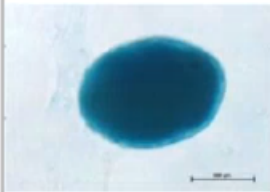
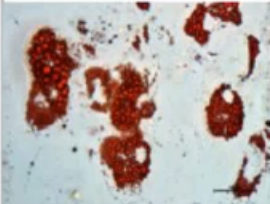
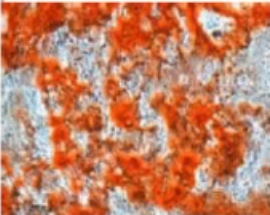
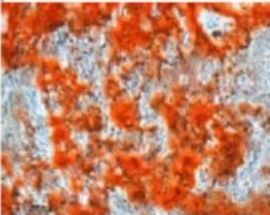
Adipocytes
Oil red O

Osteoblasts
Alizarin red

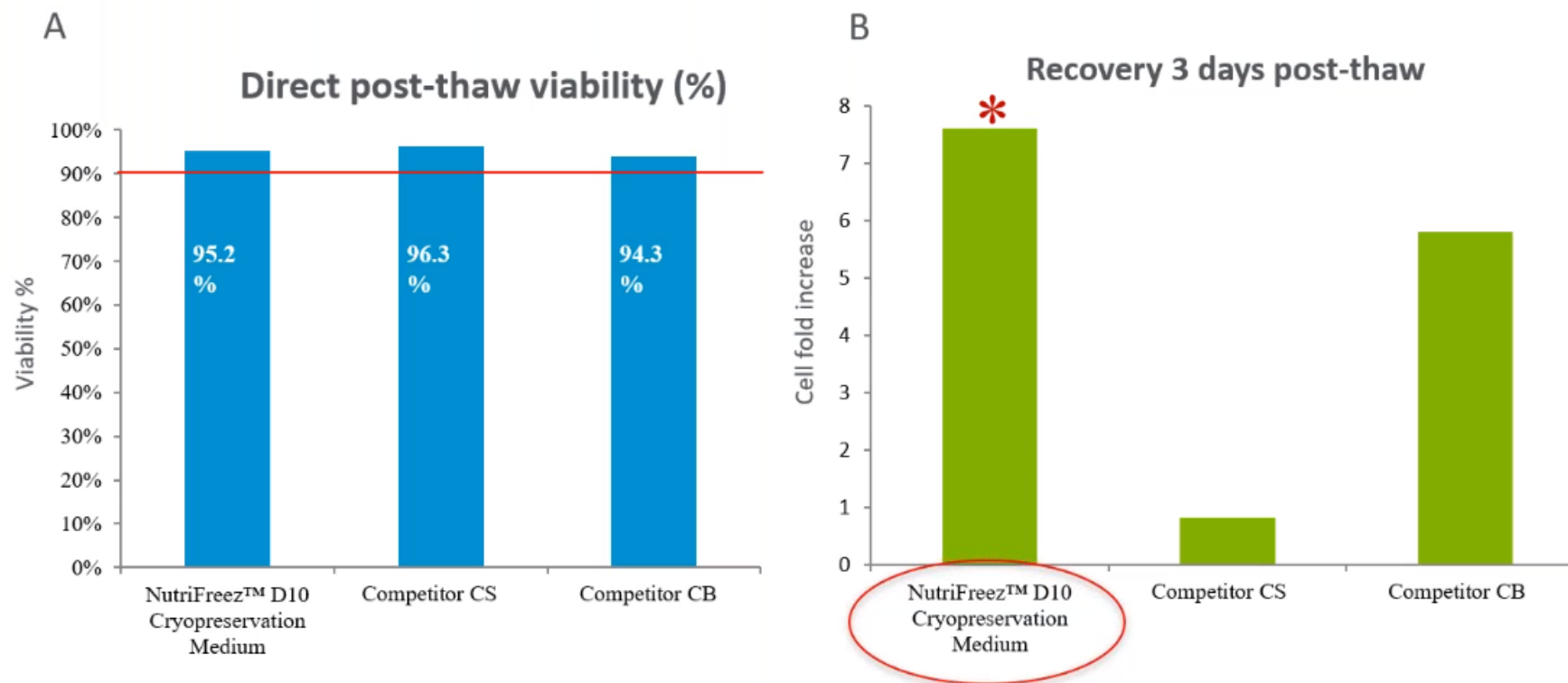
Chondrocytes
Alcian blue

hMSC cultured in MSC NutriStem[®] XF (3-5p) maintain tri-lineage differentiation potential

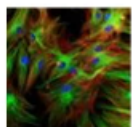
MSC NutriStem XF: Tri-lineage differentiation

MSC go XF Differentiation media (BI)				Storage Temp.	Notes
	MSCgo™ Chondrogenic XF				The complete medium is stable for 1 months at 2-8°C
	Basal	05-220-1B (100ml)	05-220-1A (500ml)	2-8°C	
	Sup. Mix.	05-221-1D (10ml)	05-221-1E (50ml)	-20°C	
	MSCgo™ Adipogenic XF				The complete medium is stable for 1 months at 2-8°C
	Basal	05-330-1B	05-330-1A	2-8°C	
	Sup. Mix.	05-331-1-01 (100ul)	05-331-1-05 (500ul)	-20°C	
	Sup. Mix.	05-332-1-15 (1.5ml)	05-332-1-75 (7.5ml)	-20°C	
	MSCgo™ Osteogenic XF				
	05-440-1B, 05-4401A a complete ready to use medium			2-8°C	
	MSCgo™ rapid Osteogenic XF				
	05-442-1B, 05-442-1A a complete ready to use medium			2-8°C	

Human Mesenchymal Stromal Cells – Cryopreservation



All Serum-Free Freezing Products yield greater than 94% viability (A) **however, hMSC-BM cells cryopreserved with NutriFreez® D10 exhibited superior recovery (B) after 3 days of growth compared to competitors, while keeping normal cell morphology (C)**



NutriFreez® D10 Cryopreservation Medium

- ✓ Chemically defined and animal component-free
- ✓ Ready-to-use, stored at 4°C
- ✓ Manufactured under cGMP conditions
- ✓ FDA Drug Master File (DMF) submitted
- ✓ Contains: Methylcellulose and 10% DMSO

Cat. No.	Size
05-713-1A	500 mL
05-713-1B	100 mL
05-713-1C	20 mL
05-713-1D	10 mL
05-713-1E	50 mL



Submitted



DMF



cGMP
Manufacturing
Facility

Summary

- hMSC from **various sources** can be efficiently isolated using MSC NutriStem® XF and MSC Attachment Solution.
- A **higher number** of hMSC was obtained after isolation using MSC NutriStem® XF in comparison to FBS-containing medium.
- Early cultures obtained from primary tissues show significantly **reduced hematopoietic cell contamination** compared to initial isolation using FBS-containing medium.
- The **highest proliferation rate** of hMSC from a variety of sources was achieved using MSC NutriStem® XF in comparison to other commercially available SF media.
- MSC NutriStem® XF **supports long-term culture** of hMSC from a variety of sources.
- hMSC cultured in MSC NutriStem® XF **maintain the essential MSC characteristics**.
- Rapid recovery and high viability of hMSC after thawing was observed when using NutriFreez® D10 Cryopreservation medium

Clinical Trials using MSC NutriStem

The Ottawa Hospital Research Institute (Canada)

First ever clinical trial of stem cells for septic shock (2016)

"The Ottawa Hospital's trial is called "**Cellular Immunotherapy for Septic Shock**," and is the result of research led by Dr. Duncan Stewart and Dr. Lauralyn McIntyre.

While mesenchymal stem cells have been used in clinical trials for other conditions, this is the first trial in the world to see how the cells specifically treat septic shock, according to the hospital's news release" *CBC News*

"In an experimental trial, researchers at the Ottawa Hospital are testing hMSC from a healthy donor in patients admitted with sepsis, a runaway infection that invades the body, leading to multiple organ failure and ultimately to septic shock. The condition is fatal in 20 to 40 per cent of cases" (CTV News, Ottawa)

<http://www.ctvnews.ca/health/ottawa-researchers-testing-stem-cells-for-septic-shock-1.2841082>

***Cell expansion with:
BI MSC Nutristem XF culture medium***



Clinical Trials using MSC NutriStem

Clinical trial using human MSC for generation of bone grafts

Bonus BioGroup (TASE: BONS) is a biotechnology company applying a proprietary, innovative technology to generate viable tissue-regenerating bone grafts. The company strives to become a world leader in the field of tissue engineering and live bone transplantation.

A **clinical trial** was launched in April 2014 to evaluate the safety and efficacy of the company's live human bone grafts for the repair of maxillofacial bone deficiency, including upper and lower jawbone cavitation. These grafts are comprised of autologous adipose (fat) tissue cells.



Cell expansion with:

BI MSC Nutristem XF culture medium

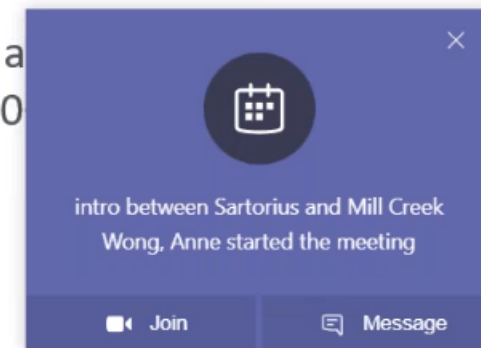
<http://reut.rs/2h70Qbe>

Bonus BioGroup LTD (Israel)



Clinical Trials using MSC NutriStem

- V.T. Hoang, et al. Expansion of Human Mesenchymal Stromal/Stem Cells Using Standardized Xeno-Free, Serum-Free Culture Condition blood, Volume 134, Issue Supplement_1, November 13 2019, <https://doi.org/10.1182/blood-2019-132140>
- C. Bozkurt, et al. The Use of Allogeneic Mesenchymal Stem Cells in Childhood Steroid-Resistant Acute Graft-Versus-Host Disease: A Retrospective Study of a Single-Center Experience tjh, 2019, 2019.0090 ;36:186-192
- D. Ben-David, et al. Autologous cell-coated particles for the treatment of segmental bone defects—a new cell therapy approach Journal of Orthopaedic Surgery and Research, volume 14, Article number: 198 (2019)
- L.A McIntyre et. al. Cellular Immunotherapy for Septic Shock. A Phase I Clinical Trial. American Journal of Respiratory and Critical Care Medicine, Vol. 197, No. 3, 2018
- K. Schlosser et al. Effects of Mesenchymal Stem Cell Treatment on Systemic Cytokine Levels in a Escalation Safety Trial of Septic Shock Patients. Critical Care Medicine, doi: 10.1097/CCM.00



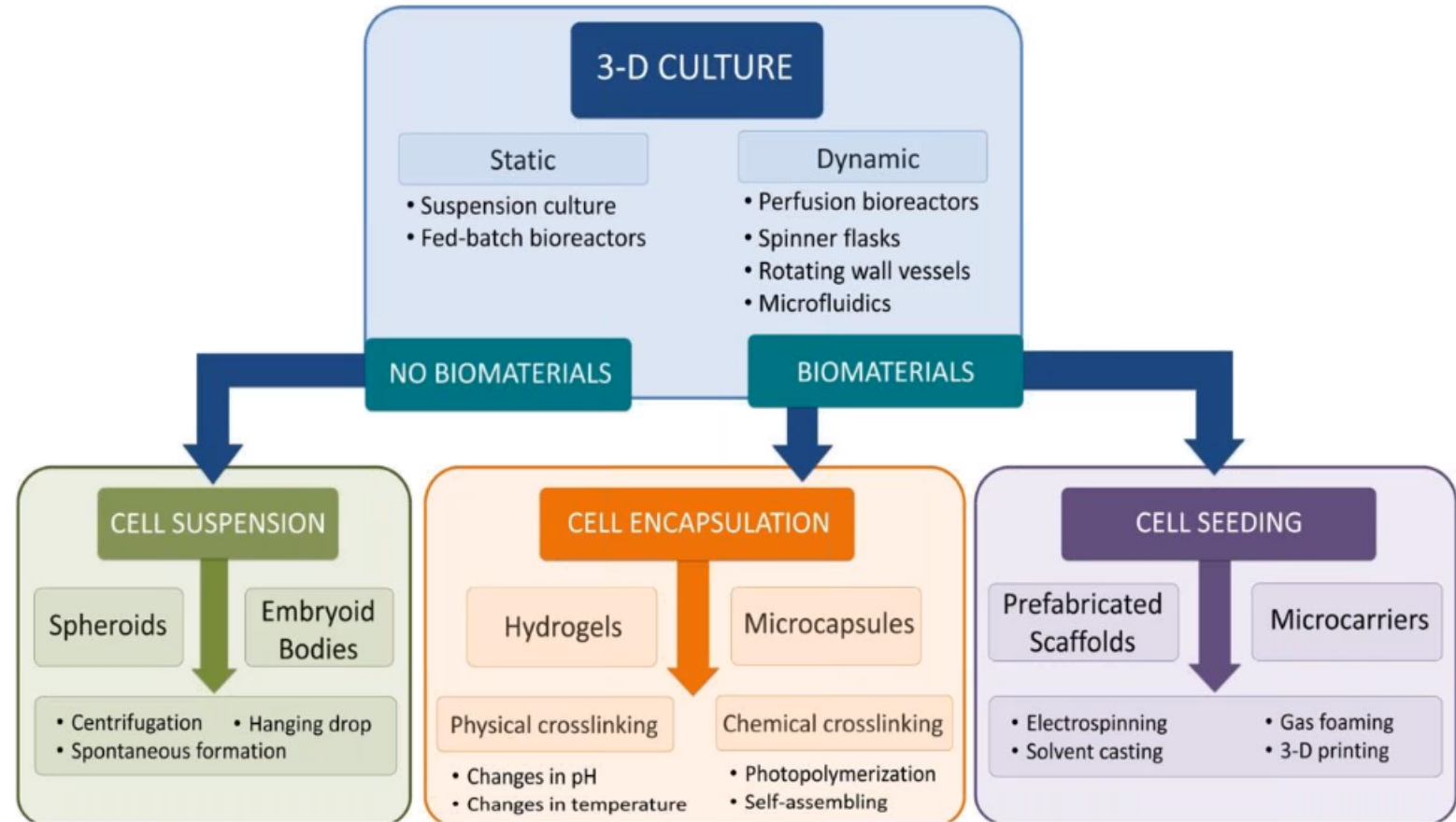
Scale up expansion of hMSCs



Highlights

- Lack of scalability of 2-D culture systems.
- Physiologically relevant human cell culture techniques.
- Techniques mimicking native microenvironment for SC growth and differentiation.
- Higher density and billion-fold expansion using 3-D culture systems.
- Challenges and advantages of 3-D culture systems.

Advances and challenges in stem cell culture
Author Christina McKeeab, G. Rasul Chaudhryab



THANK YOU

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Support@Bioind.com

www.bioind.com