Growing more cells: cGMP culture environments for high yield expansion of MSCs

Application Note

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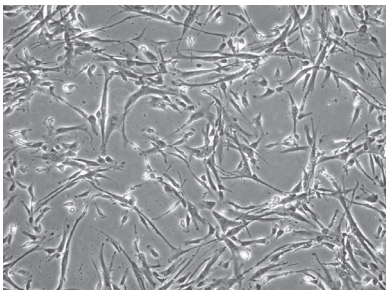
Abstract

Mesenchymal stem/stromal cells (MSCs) have been found to be broadly applicable in the emerging field of regenerative medicine as well as in cellular therapies; however, these cells are present in minute quantities in vivo and therefore must be expanded in vitro to generate enough cells to be useful therapeutically. When expanding large numbers of cells in vitro for translational use, a reliable and consistent culture system that is of clinical quality, cGMP-produced, and supportive of healthy, long-term proliferation of high-quality MSCs is extremely important. Additionally, the culture method must also be cost-effective such that it may be applied to various scientific and therapeutic workflows. Here we present Biological Industries' (BI) NutriStem® MSC Medium, a serum-free, xeno-free MSC culture medium that can be used with and without human platelet lysate for exceptional and dependable proliferation of MSCs, capable of facilitating growth rates greater than 10-fold per passage, which could support MSC expansion from less than one million cells to hundreds of millions of cells in one to two weeks. In comparison, MSC culture in traditional media supplemented with FBS can take over a month to achieve similar cell yields or require a much larger starting population to match this two-week expansion time frame. The NutriStem® MSC Medium's ability to support excellent expansion of high-quality cells in an efficient and cost-effective manner is of significant benefit to researchers in all phases of translational research.



BM-MSCs cultured in NutriStem® MSC Medium

Introduction

MSCs are multipotent cells capable of regenerating numerous tissue types (e.g. bone, cartilage, fat, and muscle) as treatments for orthopedic injuries, cardiovascular disorders, liver disease, and other tissue engineering applications. Beyond this, MSCs have been utilized in cancer therapies, wound healing, and immune regulation.[1] These tissue-specific progenitor cells have been isolated from a variety of tissues, including bone marrow, adipose tissue, umbilical cord, placenta, and dental pulp, yet the occurrence of these cells in vivo is typically low.[2,3] For example, MSCs only make up approximately 0.01-0.001% of cells present in bone marrow aspirate.[4] Since an average therapeutic dose of MSCs can potentially require hundreds of millions of cells, the need for healthy cell proliferation and expansion in vitro is critical. Additionally, the health and quality of MSCs must be maintained in such a way to generate enough cells before they senesce, or lose their multipotentiality, as these cells are known to do in long-term culture.

Historically, MSCs have been cultured in a classical basal medium supplemented with animal serum. Fetal bovine serum (FBS) is traditionally the most widely used protein supplement added to MSC culture media, yet it is wrought with large lot-to-lot variation and the risk of transmitting infections or initiating xenogeneic immune responses.[5] In addition, the FBS market is not equipped to meet the ever-increasing demand for high-quality serum, creating an unstable supply and expensive, highly fluctuating prices. As interest in clinical translation of MSCs increases, so does the need to generate clinical-quality MSCs in a serum-free environment for more controlled and reproducible culture conditions.

Biological Industries' NutriStem® MSC Medium can be used to produce clinical-quality MSCs, either alone or in combination with protein-rich human platelet lysate. NutriStem® MSC Medium is a completely defined, serum-free, xeno-free, low-protein formulation. Due to the low concentrations of protein content within the defined medium, MSCs require the addition of a substrate for proper attachment and expansion in culture. MSCs expanded in the NutriStem® MSC Medium were cultured on human fibronectin, maintaining a serum-free, xeno-free culture environment (noted here as System 1).

NutriStem® MSC Medium can also be supplemented with 5% PLTGold® Human Platelet Lysate (noted here as System 2) for excellent expansion in culture. PLTGold® Human Platelet Lysate contains sufficient proteins and cytokines to support exceptional MSC attachment and proliferation without the need for any attachment substrate. Both NutriStem® MSC Medium and PLTGold® Human Platelet Lysate are clinical-grade, manufactured under cGMP, and have specific Drug Master Files (DMFs) on record with the FDA.

In this paper we present NutriStem® MSC Medium as part of two culture systems that each facilitate high-quality MSC growth in culture, uniquely enabling large-scale expansion that outperforms FBS and better supports proliferation of clinical-quality MSCs. Combined with PLTGold® Human Platelet Lysate, NutriStem® MSC Medium allows for accelerated cell expansion timelines, enabling over 200 million MSCs to be generated in less than two weeks.

NutriStem® MSC Medium

Materials and Methods

Cell Culture and Conditions

Human bone marrow-derived mesenchymal stem cells (StemExpress® Human BM-MSCs, Biological Industries USA, BMMSC001C) were thawed rapidly at 37°C and plated into the two NutriStem® MSC Medium culture systems defined in Table 1.

Culture System Comparison	System 1 NutriStem® MSC Medium on Human Fibronectin	System 2 NutriStem® MSC Medium + PLTGold® Human Platelet Lysate	FBS Classical Media + 10% FBS
Application example	MSC Exosome Isolation	Stem Cell Banking	Standard for Basic Research
Defined	●	X	X
Xeno-free	●	●	X
Lot-to-lot consistency	●	●	X
Protein-rich	X	●	●
Substrate required	●	X	X
GMP	●	●	X
FDA DMF	●	●	X
Clinical-grade	●	●	X

Table 1: Components and comparison of MSC culture systems. The NutriStem® MSC Medium can be used to support two clinically applicable culture systems. System 1 consists of NutriStem® MSC Medium, a completely defined, serum-free, low-protein medium, which requires the use of a human fibronectin protein substrate for the attachment and culture of cells. In System 2, NutriStem® MSC Medium is supplemented with PLTGold® Human Platelet Lysate, providing sufficient proteins and cytokines for MSC proliferation and expansion without the need for an attachment substrate. Traditional FBS-based media listed for comparison.

MSC Expansion and Characterization

Upon thaw, the StemExpress® Human BM-MSCs were seeded at 2.6 x 10^3 cells/cm^2 onto 6-well plates for culture in both Systems 1 and 2 and were incubated at 37°C in a humidified atmosphere of 5% CO_2. Seeding density optimization for subsequent passages was determined during continued culture. Cells were seeded at densities ranging between 8 x 10^2 to 6.3 x 10^3 cells/cm^2 and these cultures were maintained in each system with media changes every other day.

After three passages (11 days), cells cultured in System 2 were analyzed via flow cytometry for the presence of multipotent stromal (CD73, CD90 and CD105) and non-stromal (CD14, CD20, CD34 and CD45) cell surface markers. Cells cultured in both systems were maintained for a total of five passages (20 days), during which time cell expansion (fold increase) was measured by hemocytometer-based cell counting at every passage. Using the growth rates observed through five passages, expected MSC yields were extrapolated for both systems and compared to the cell yields achieved from growth rates typical of traditional MSC culture with FBS (average 2.5-fold increase per passage).

Results

Optimal Seeding Density

The timeline required to reach 70 to 80% confluency was dependent on the system used and demonstrated an inverse relationship between initial seeding density and the amount of days required before passaging. Cultures seeded at a density within the range recommended by Biological Industries' protocols (2.0 x 10^3 to 5.0 x 10^3 cells/cm^2) were able to achieve 70 to 80% confluency in 4 days (Figure 1, solid blue outline). In comparison, a higher seeding density (6.3 x 10^3 cells/cm^2) required less time (3 days) to reach an appropriate density for passaging, while lower seeding densities increased the amount of time required before passaging to 5 days or more. At the lowest density tested (8.0 x 10^2 cells/cm^2), only cells cultured in System 2 were able to reach 70 to 80% passaging confluency in less than 7 days.

While a variety of factors, including cell isolation source, culture health, and passage number, can affect MSC culture and growth rates, the data presented in Figure 1 provides a frame of reference for determining the ideal seeding density needed to obtain 70 to 80% passaging confluency at a desired time interval when using a respective NutriStem® MSC Medium culture system.

Culture System Comparisons for Seeding Densities

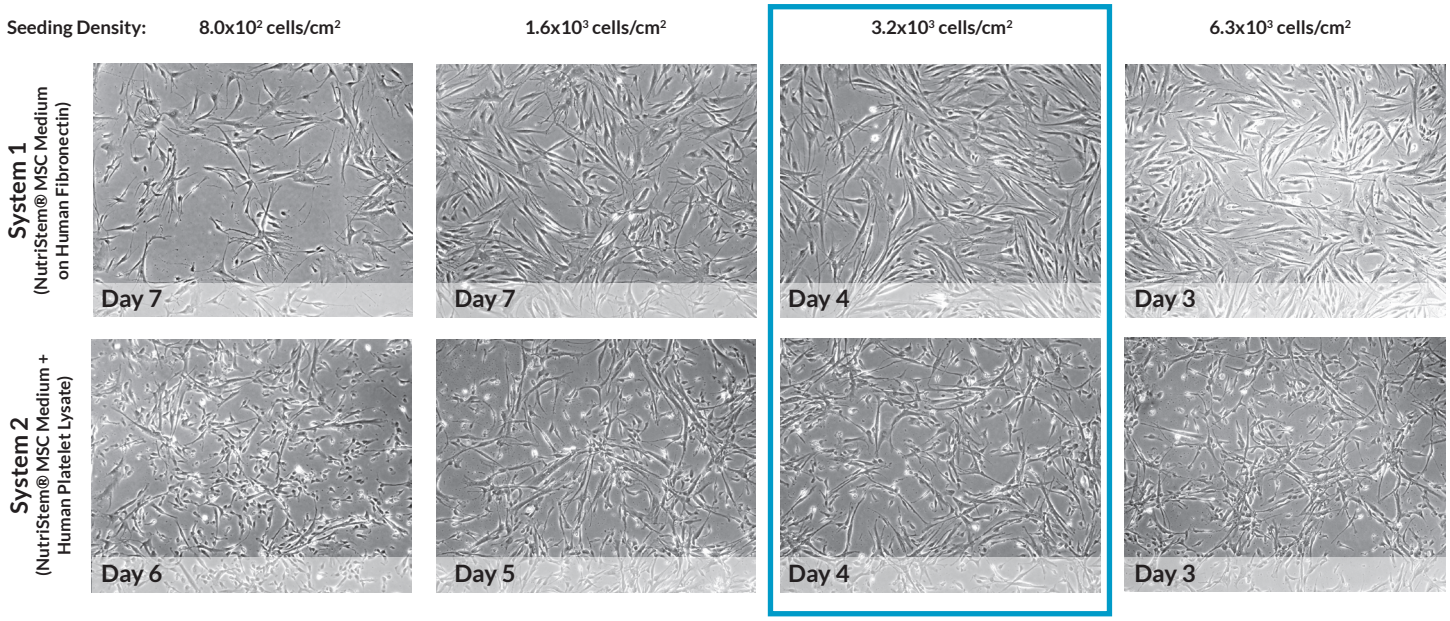


Figure 1: Impact of MSC seeding density on the time required to reach passaging confluency. StemExpress® Human BM-MSCs were seeded into each of the two culture systems at seeding densities ranging from 8.0 x 10^2 to 6.3 x 10^3 cells/cm^2 to determine optimal MSC seeding density. The number of days required to reach 70 to 80% passaging confluency was noted for both systems used. For both culture systems, MSCs plated at 3.2 x 10^3 cells/cm^2 consistently reached 70 to 80% passaging confluency in 4 days (solid blue outline).

MSC Characterization

MSCs cultured in System 2 were evaluated for the presence and absence of MSC-specific cell surface markers at Passage 3. Flow cytometric analysis revealed that the expanded MSCs retained high viability (98.61%) during expansion and were highly positive for multipotent stromal markers (CD73: 99.95%, CD90: 98.16%, and CD105: 99.71%), each exceeding the International Society for Cellular Therapy's (ISCT) minimum standard of 95%. These results, combined with low expression of non-stromal markers (CD14/20/34/45) at 4.41% (Figure 2), confirm that NutriStem® MSC Medium supplemented with PLTGold® Human Platelet Lysate supports the expansion of high-quality, phenotypically pure MSCs.

Cell Marker Expression of BM-MSCs

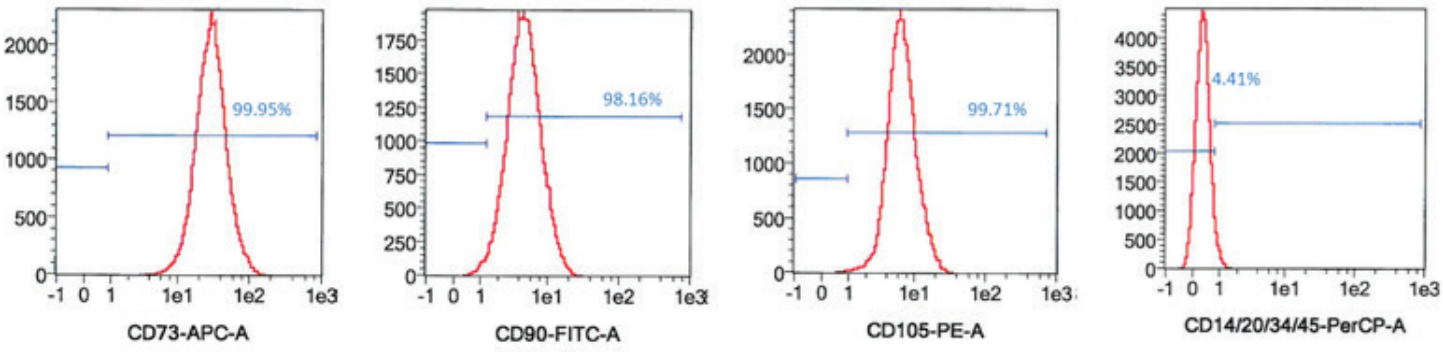


Figure 2: Flow cytometric analysis of BM-MSCs expanded in NutriStem® MSC Medium supplemented with 5% PLTGold® Human Platelet Lysate (System 2). StemExpress® Human BM-MSCs were thawed and cultured through Passage 3 (11 days) in System 2. While cells expanded in NutriStem® MSC Medium and PLTGold® proliferate rapidly, phenotypic assessment by flow cytometry show that the cells in culture maintain the marker expression profile of high-quality MSCs.

MSC Growth Rates and Expansion Yields

Through five passages (20 days), both NutriStem® MSC Medium culture systems delivered consistent growth rates and higher cell yields at each passage than expected yields for MSCs cultured in traditional MSC media containing FBS (Figure 3). While System 1 yielded a 4-fold average increase in cell number at each passage, System 2 yielded an average increase that was greater than 10-fold at each passage (Table 2). Cells in both NutriStem® MSC Medium systems were passaged every 4 days when the cultures reached 70 to 80% confluency. When the observed cell culture growth rates are used to estimate the total cell yields for the expansion of a starting cell population of 875,000 MSCs, System 1 is expected to yield 1.0 x 10^9 MSCs by Passage 5, while System 2 would yield 2.5 x 10^{11} MSCs by Passage 5 (Figure 3).

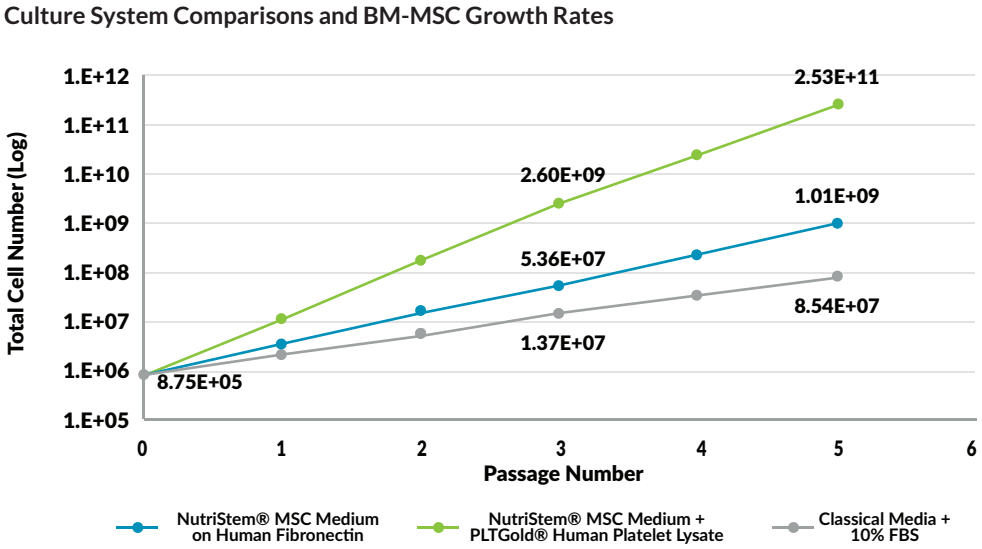


Figure 3: MSC growth rates and estimated cell yields in the NutriStem® MSC Medium culture systems in comparison to traditional media using 10% FBS. Using growth rates measured out to Passage 5, estimated cell yields were calculated at each passage assuming a starting value of 875,000 MSCs in each system. For expansion of MSCs in traditional culture with FBS-containing media, cell yield estimates were calculated assuming a 2.5-fold expansion over seven days in culture (the time typically required for cells to reach 70 to 80% confluency in FBS-based media).

These higher cell yields at earlier passages translate to both time and cost savings. A simple flask culture method** typical of protocols for the initial expansion of therapeutic doses (up to 50 million MSCs)[6,7,8] would require greater than four weeks of culture time using traditional FBS-containing media. Alternatively, the NutriStem® MSC Medium culture systems are expected to yield equivalent quantities of MSCs in as little as one to two weeks, notably at a significantly lower cost for System 2 (Table 2). Additional scale-up from 50 million to 200 million or more cells typically utilizes a bioreactor or multi-layer flasks which further improve yield and shorten the time to generate a clinical dose of cells.

	Culture System Comparisons	System 1 NutriStem® MSC Medium on Human Fibronectin	System 2 NutriStem® MSC Medium + PLTGold® Human Platelet Lysate	FBS Classical Media + 10% FBS
	Fold increase per passage	4	13	2.5
First passage to ≥50 million cells	**Passage (time in culture)**	P3 (12 days)	P2 (8 days)	P5 (35 days)
First passage to ≥50 million cells	**Actual cell yield at passage**	5.4×10^7	1.8×10^8	8.5×10^7
Calculated effort to reach 50 million cells	**Total media required**	1,932 mL	420 mL	5,040 mL
Calculated effort to reach 50 million cells	**Total T-175 flasks used**	23	5	40
Calculated effort to reach 50 million cells	**Approximate media cost***	$1,400	$350	$1,110

**Materials and costs are estimated based on current USD list pricing.*

Table 2: Expansion potential and cost comparison of NutriStem® MSC Medium culture systems and traditional classical media supplemented with 10% FBS. Using observed growth rates, the first passage to generate more than 50 million cells from an initial starting amount of 875,000 cells was calculated. This growth rate was then used to extrapolate the volume of media components required to generate 50 million cells.** Using System 2, an actual yield of 180 million cells could be achieved by Passage 2, therefore the time required to reach 50 million cells is less than 8 days. The defined NutriStem® MSC Medium on human fibronectin generates the same number of cells in about half the amount of time, materials, and reagents than media supplemented with FBS. Pairing NutriStem® MSC Medium with PLTGold® enables even more rapid cell proliferation, generating 50 million cells within one week, and only requiring 5 culture flasks.

Discussion

The number of therapies utilizing adult stem cells in a clinical setting is rapidly increasing. However, many traditional MSC expansion methods lack definition, are costly, and are not time efficient. Biological Industries has developed the optimized, cGMP-compliant, clinical-grade NutriStem® MSC Medium that supports high-yield expansion rates of high-quality MSCs, and can be used as either a complete medium or in combination with PLTGold® Human Platelet Lysate – both systems demonstrating unique benefits over MSC culture with traditional FBS-containing media.

NutriStem® MSC Medium (System 1) facilitates expansion of MSCs in a completely defined medium at a growth rate nearly twice that of typical MSC cultures containing FBS, achieving steady proliferation of MSCs in a serum-free, xeno-free environment. This system may be ideal for research requiring a minimal medium composition, such as MSC exosome isolation and purification.[9] The addition of PLTGold® Human Platelet Lysate to NutriStem® MSC Medium (System 2) enables MSC growth rates greater than 10-fold per passage, which could support MSC expansion from less than one million cells to hundreds of millions of cells in as little as one to two weeks of culture. For cell banking, MSC culture scale-up, and other therapeutic applications, System 2 may be more beneficial in providing the highest possible clinical-quality cell yield in a rapid time frame. The NutriStem® MSC Medium culture systems are capable of providing faster growth rates and higher MSC yields in a completely defined medium at a cost similar to that of typical FBS-based MSC culture media (System 1) or in a protein-rich system at a fraction of the cost of typical FBS-based MSC culture media (System 2), providing researchers with a completely customizable environment based on their culture and application requirements.

Product List	Cat. No.	Qty
StemExpress® Human BM-MSCs, Frozen	BMMSC001C	1 million cells
NutriStem® MSC XF Medium (Basal Medium and Supplement)	05-200-1A-KT	500 mL
MSC Attachment Solution (Human Fibronectin)	05-752-1F	1 mL
PLTGold® Human Platelet Lysate	PLTGOLD100R	100 mL
Recombinant Trypsin Solution	03-078-1B	100 mL
Soybean Trypsin Inhibitor (SBTI)	03-048-1C	20 mL
DPBS (without Ca & Mg)	02-023-1A	500 mL
Trypan Blue (0.5% Solution)	03-102-1B	100 mL

**Culture parameters used to estimate media volumes and total costs: a seeding density of 5 x 10^3 cells/cm^2, cultured in 175cm^2 flasks, with 42 mL complete media used per flask, and passaging (sub-culture) performed at time required to reach 70 to 80% confluency for each media condition (4 days for both NutriStem® MSC Medium culture systems and 7 days for traditional culture with FBS).

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For additional product or technical information, please visit the Biological Industries USA website at www.bioindusa.com, email techsupport@bioindusa.com, or call customer service at 1-860-316-2702.