

# myMATRIX MSC – Chemically Defined and Extracellular Matrix-mimetic Cultureware for Rapid hMSC Expansion

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## Abstract

Human mesenchymal stromal cells (hMSC) have the potential for massive impact in the fields of cell-based therapy, tissue engineering and regenerative medicine because of their ready availability, stem cell properties and immunomodulatory activity. Prior to their use for research or clinical applications, hMSC must be expanded in order to reach sufficient numbers of cells without reducing their homing ability, multilineage differentiation potential and immunomodulatory properties.

To create optimal growth conditions for hMSC, *in vitro*-specific aspects of the *in vivo* microenvironment need to be mimicked. The novel chemically defined, animal and human component-free coating of myMATRIX MSC combines biologically relevant synthetic peptides with sulfated polysaccharides to facilitate cell adhesion and promote cell expansion. This ready-to-use consumable is optimized for serum-free culture of hMSC. Bone marrow-derived hMSC cultured on myMATRIX MSC show enhanced proliferation while maintaining their characteristic cell morphology,



high viability, typical surface marker expression profile and the ability to differentiate *in vitro* into the three mesoderm lineages.

In addition, myMATRIX MSC supports the long-term culture of adipose-derived and umbilical cord-derived hMSC. This ready-to-use surface enhancing lot-to-lot consistency and reliable performance.

## Introduction

Mesenchymal stromal cells (MSC), also termed human mesenchymal stem cells, are a prominent subject in research as well as in cell-based therapies due to their clinical relevance and biological importance. Based on their capacity to initiate tissue regeneration and their immunomodulatory activity they are considered as promising biological tool for regenerative medicine as well as for the treatment of immune diseases (1, 2).

MSC are a heterogeneous population of multipotent, self-renewing stromal cells. Within the human body, they are present throughout lifetime in numerous stem cell niches. MSC were first described in the 1970s by Friedenstein and colleagues as proliferating adherent colonies of fibroblastoid cells from a human bone marrow cell suspension (3). Today, they can be isolated from adult tissue such as bone marrow, peripheral blood, lung, skin, muscle and adipose tissue, as well as from fetal/neonatal sources

such as placenta, amnion, umbilical cord or cord blood (4).

Adipose-derived MSC are easily isolated and are considered a vital source of MSC for tissue regeneration while umbilical cord tissues offer the greatest number of harvestable MSC.

Depending on the tissue source, the isolation and culture procedure MSC may feature different characteristics. Due to that and because they share many features with fibroblasts including morphology and surface marker expression, the International Society for Cellular Therapy has defined minimal criteria for MSC: (1) *ex-vivo* plastic-adherent growth abilities in standard culture conditions, (2) expression of cell surface antigens CD105, CD73 and CD90, while lacking the expression of CD45, CD34, CD14, or CD11b, CD79 $\alpha$  or CD19 and HLA-DR, (3) *in vitro* differentiation into osteoblasts, adipocytes and chondroblasts (5).

### ***hMSC culture conditions***

MSC are a very rare population in their tissue of origin. Thus, to reach adequate numbers of high-quality cells for research and clinical applications optimal growth conditions for MSC *in vitro* are needed. In order to provide sufficient amounts of nutrients and growth factors to promote cell survival and growth, supplementing media with fetal bovine serum is still common practice in cell culture applications. However, the use of serum also bears a number of disadvantages such as unknown composition, batch-to-batch variation, risk of infectious agents and contaminants. Due to the physiological variance of serum, it may also impede the reproducibility of research results or lead to their misinterpretation. Moreover, using serum-containing medium limits the impact of research results with regard to clinical applications (6).

In the last decades, there have been increasing efforts towards the establishment of serum-free as well as xeno-free and chemically defined cell culture expansion processes (7). To achieve this, specific aspects of the *in vivo* microenvironment such as attachment molecules need to be mimicked *in vitro*. As adherent cells, MSCs require these molecules on the culture surface, part of which is provided by serum and thus missing in serum-free conditions. Hence, MSC often show altered adhesion characteristics, morphology, and proliferation when cultured in serum-free media. To avoid this, pre-coating cell culture plasticware with extracellular matrix protein extracts such as fibronectin are often recommended by media manufacturers.

**myMATRIX MSC**

A recently developed strategy enables the use of a chemically defined surface coating for the preparation of controlled and reproducible cell culture conditions. Based on a proprietary coating technology, denovoMATRIX' myMATRIX MSC recreates essential elements of the extracellular matrix of the human body using biomimetic peptides and complex polysaccharide molecules. The peptides include an arginine-glycine-aspartate (RGD) motif that facilitates cell adhesion by stimulating integrin receptors of the cells. The complex polysaccharide molecules mimic the function of glycosaminoglycans (GAGs). *In vivo*, GAGs are critical in the maintenance of growth factor stores and the stabilization of ligand-receptor interactions. Thus, myMATRIX MSC replicates the *in vivo* environment more reliably than existing technologies and creates optimal growth conditions for human MSC (hMSC). The myMATRIX MSC is suitable for long-term expansion of bone marrow-derived hMSC, and is compatible with various commercial dissociation reagents, supplements, serum- and xeno-free media. hMSC cultured on this novel chemically defined coating for ten consecutive passages show enhanced proliferation while maintaining their characteristic cell morphology, high viability, typical hMSC surface marker expression profiles and the ability to differentiate *in vitro* into osteoblasts, adipocytes and chondrocytes, representing the three mesoderm lineages.

## Ordering information

Description	Size	Order no.	Link
screenMATRIX	96 well plate	S1001	<a href="https://www.denovomatrix.com/products/screenmatrix">https://www.denovomatrix.com/products/screenmatrix</a>
myMATRIX MSC	T75	C0601	<a href="https://www.denovomatrix.com/products/mymatrix-msc">https://www.denovomatrix.com/products/mymatrix-msc</a>
	T25	C0701	
	24 well plate	C0301	
	6 well plate	C0501	

## Materials and Methods

### Cell culture

Two different lots of bone marrow-derived hMSC were used for this study: Lot 18TL113327 and Lot 18TL312488 (Lonza, #PT-2501), hereafter referred to as Lot 1 and 2, respectively.

Thawed cells were seeded in tissue culture treated (TCT) flasks (25 cm<sup>2</sup>, TPP, #90026) at a density of 2.800 cells/cm<sup>2</sup> and cultured for 24 h in MSCGM medium (Lonza, #PT-3001) either on myMATRIX MSC or on tissue culture-treated plastic in standard cell culture conditions (37°C, 5% CO<sub>2</sub>, humidified atmosphere). Thereafter, the medium was changed either to serum-free (CTS™ StemPro™ MSC SFM (Gibco, A1033201) + glutamine (2 mM, Merck Millipore, #K0283)) or medium with serum (DMEM (low glucose, GlutaMAX supplement, pyruvate, Thermo Fisher Scientific, #21885025) + 10% fetal calf serum (FCS, Sigma Aldrich, #F7524)) resulting in the following experimental conditions: 1) myMATRIX MSC/serum-free, 2) myMATRIX MSC/with serum, 3) plastic/serum-free, 4) plastic/with serum.

Adipose-derived (ATCC, #PCS-500-011) and umbilical cord-derived (ATCC, #PCS-500-010) hMSC were expanded in DMEM + 10% FCS prior to long-term expansion in the four experimental conditions.

Upon reaching 70-80% confluence, cells were harvested using 0.05% Trypsin-EDTA and subcultured into the same conditions for the multi-passage study. Viability and cell number were assessed using Trypan Blue dye exclusion with an automatic cell counter (EVE, NanoEnTek, #10027-452). A full media exchange was performed on day 3 or 4 only if additional time in culture was needed to meet the desired confluence for harvest and passaging. Cell morphology, viability, cell number and population doublings were assessed at each passage for all experimental conditions.

The following formula were used to calculate the population doublings (PD) and doubling times (DT):

$$PD = 3.321 (\log N - \log N_i)$$

$$DT = \text{time in culture (hours)} \times \frac{1}{\ln(2)/\ln(N/N_i)}$$

N = total number of viable cells harvested

N<sub>i</sub> = initial number of cells seeded

### Flow cytometry

The expression of positive hMSC-specific surface marker was determined at passage two, five and twelve for each experimental condition. The expression of CD90, CD73, CD105, CD146, CD44 and CD166 (see table 1) was evaluated using Attune NxT flow cytometer. For each marker, the corresponding isotype control was prepared to measure non-specific staining. Data analysis was performed using Attune NxT analyzing software.

### In vitro multilineage differentiation assay

The multipotent differentiation potential was evaluated using the StemPro Adipogenesis, Osteogenesis and Chondrogenesis Differentiation Kits (Thermo Fisher Scientific, #A1007001/ A1007101/ A1007201). Briefly, for adipogenesis and osteogenesis cells were seeded at a density of 10.000 cells/cm<sup>2</sup> in 24 well plates. After reaching confluence, the medium was replaced by the corresponding complete medium. Medium exchange was performed twice a week. For chondrogenic differentiation cell micromass cultures of 1.6 x 10<sup>7</sup> cells were prepared and seeded as 5 µl droplets in the center of each well. After two hours of cultivation complete chondrogenesis differentiation medium was added and exchanged every 2-3 days. At the end of differentiation (14 – 21 days) the extent of differentiation was determined microscopically, either by the appearance of Oil Red O-stained lipid vacuoles in adipocytes, Alizarin Red-stained calcium deposits produced by osteocytes or Alcian Blue-stained proteoglycans synthesized by chondrocytes.

Table 1. Primary antibodies and isotype control.

Antibody	Company	Cat No
APC mouse anti-human CD73	Miltenyi Biotec	130-095-183
PE mouse anti-human CD90	Miltenyi Biotec	130-095-400
PE mouse anti-human CD105	Thermo Fisher Scientific	MHCD10504
PE mouse anti-human CD44	BD Bioscience	555479
PE mouse anti-human IgG2b kappa	BD Bioscience	555743
PE mouse anti-human IgG1 kappa	eBioscience	12-4714-42
APC mouse anti-human IgG1 kappa	eBioscience	17-4714-42

## Results and Discussion

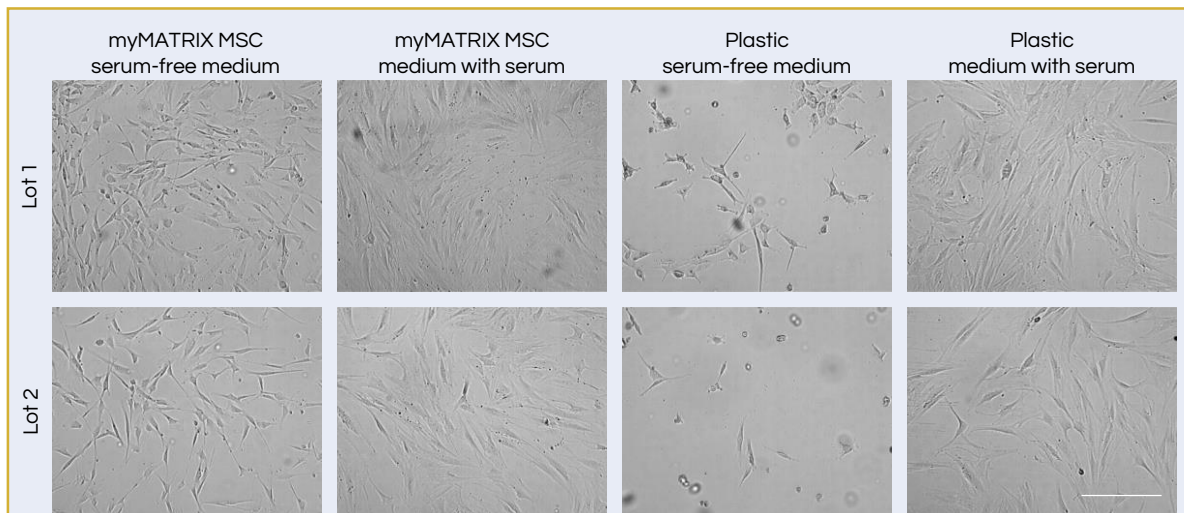
### *hMSC cultured on myMATRIX MSC maintain their characteristic morphology*

Cell morphology is an integral aspect of the phenotype of a cell and is defined by its cytoskeleton. Changes in cell morphology are not only connected to external stimuli but also coordinated with cell growth or apoptosis. Thus, the cell shape provides a quick and easy readout of the organizational and physiological state of the cell population.

The morphology of cells of both lots was evaluated after 6-8 population doublings (PD). hMSC cultured on myMATRIX MSC maintained their characteristic fibroblast-like morphology (Fig. 1). As previously described in the literature and by media suppliers for cells expanded in serum-free conditions, cells of both lots cultured on myMATRIX MSC also acquired a more compact, spindle-shaped morphology. In contrast, cells grown in medium with serum develop a flattened and spread shape.

In the absence of serum on TCT plastic surface, a small percentage of cells loosely attached as single cells or small cell clusters, with little to no spreading.

Of note, hMSC cultured on myMATRIX MSC in serum-free conditions showed lot-dependent morphological differences during the study. While Lot 1 cells maintained the more compact morphology during the entire study, cells of Lot 2 showed a tendency to develop into more elongated and stretched cells. No morphological changes were observed when hMSC were cultured on myMATRIX MSC in the presence of serum. hMSC expanded on myMATRIX MSC maintain their fibroblast-like morphology in serum-free as well as serum-containing conditions.

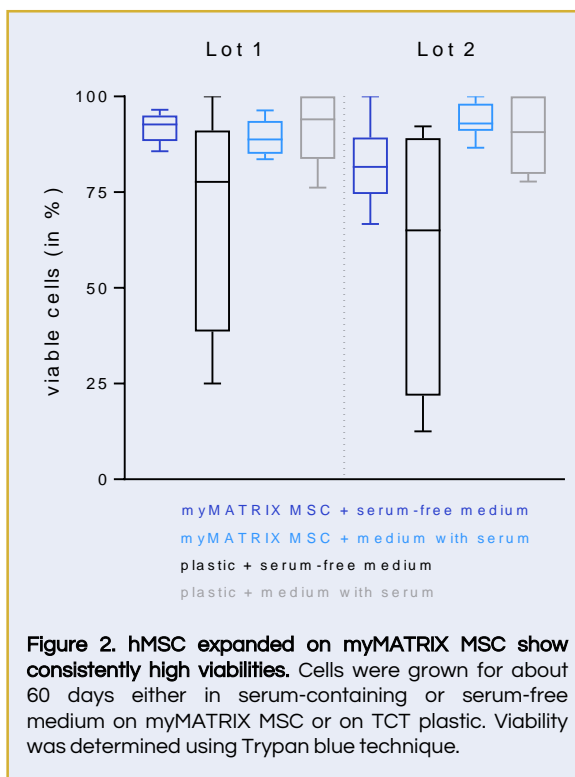


**Figure 1. hMSC cultured on myMATRIX MSC display characteristic fibroblast-like morphology.** Representative cell morphology micrographs (PD 6-8; phase contrast, 20x) for the indicated surface/media conditions are shown. hMSC cultured in traditional serum-containing medium on myMATRIX MSC or TCT plastic display their characteristic fibroblast-like morphology. myMATRIX MSC also supports attachment and growth in serum-free conditions with a more compact, spindle-shaped morphology. Due to the absence of attachment molecules in serum-free medium in combination with TCT plastic cultureware, only few cells loosely attach in these conditions. Scale bare indicates 250  $\mu\text{m}$ .

### *myMATRIX MSC supports consistently high viabilities*

*In vitro* cell viability is influenced by many factors such as media, supplements, dissociation reagents and surface adhesion. Suboptimal growth conditions and cytotoxic effects eventually lead to cell death, which negatively influences the proliferation and viability of the remaining population.

Throughout the long-term culture on myMATRIX MSC, the cells maintained high viability irrespective of the lot and condition (Fig. 2). The average viability of hMSC expanded on myMATRIX MSC was 81-93% compared to 58-88% on TCT plastic. In addition, the viability appears to be more stable on myMATRIX MSC as demonstrated by a smaller spread of data.



### *Expansion of hMSC on myMATRIX MSC results in enhanced cell proliferation*

Next, we examined if the myMATRIX MSC surface can support long-term culture of hMSC in serum-free medium. Cells from two different donors (Lot 1 and Lot 2) were expanded for 10 consecutive passages (about 60 days) using the following experimental conditions: 1) myMATRIX MSC/serum-free, 2) myMATRIX MSC/with serum, 3) plastic/ serum-free, 4) plastic/with serum (traditional culture system).

Cumulative cell numbers were calculated across multiple passages for both lots. As demonstrated in figure 3, 2-fold higher cell numbers were observed on myMATRIX MSC compared to TCT plastic surface in serum-containing conditions (light blue vs grey). In serum-free conditions, final cell numbers (table 2) were increased by the factor of 4-6 on myMATRIX MSC compared to TCT plastic (day 57; Fig. 3, dark blue vs black). Interestingly, cells of Lot 1 showed a 1.8-fold higher cell number on myMATRIX MSC in serum-free conditions than in the presence of serum after 57 days.

In contrast, cells of Lot 2 displayed better growth properties on myMATRIX MSC in medium with serum (1.5-fold increase compared to serum-free after 57 days).

Consistent with the higher cell numbers, hMSC grown on myMATRIX MSC had higher population doubling numbers compared to cells on TCT plastic (Fig. 4A). Although cells of Lot 1 and 2 showed different growth properties over time, expansion of both lots on myMATRIX MSC in serum-free medium resulted in the highest cumulative population doublings (CPD) during the first 15 days (Fig. 4A, dark blue). When grown in serum-containing conditions, cells on myMATRIX MSC showed a higher CPD than those grown on TCT plastic during the study (Fig. 4A, bright blue vs. grey). Thus, hMSC had an advantageous growth rate on myMATRIX MSC in both serum-containing and serum-free media. Cells in serum- and coating-free conditions were not able to attach properly and therefore could not be expanded until the end of the study.

Table 2. Final cumulative cell number ( $\times 10^5$ ) for the indicated surface + media.

Lot 1				Lot 2			
myMATRIX MSC serum-free medium	myMATRIX MSC medium with serum	Plastic serum-free medium	Plastic medium with serum	myMATRIX MSC serum-free medium	myMATRIX MSC medium with serum	Plastic serum-free medium	Plastic medium with serum
105.2	58.4	18.1	28.5	28.6	42.5	7.3	20.7

The enhanced proliferation of hMSC on myMATRIX MSC is also demonstrated by shorter doubling times, irrespective of the lot and medium (Fig. 4B). The median of the doubling time for cells grown on myMATRIX MSC is 33-62 h versus 56-86 h for those expanded on TCT plastic (table 3). Again, the shorter doubling times seemed to be stabilized by the myMATRIX MSC surface as indicated by a smaller spread of the data (16-147 h on coating vs. 0-436 h without coating).

In summary, culturing hMSC on myMATRIX MSC in tandem with serum-free media results in enhanced proliferation with higher cell numbers and shorter doubling times compared to cells expanded in traditional serum-containing culture system on TCT plastic.

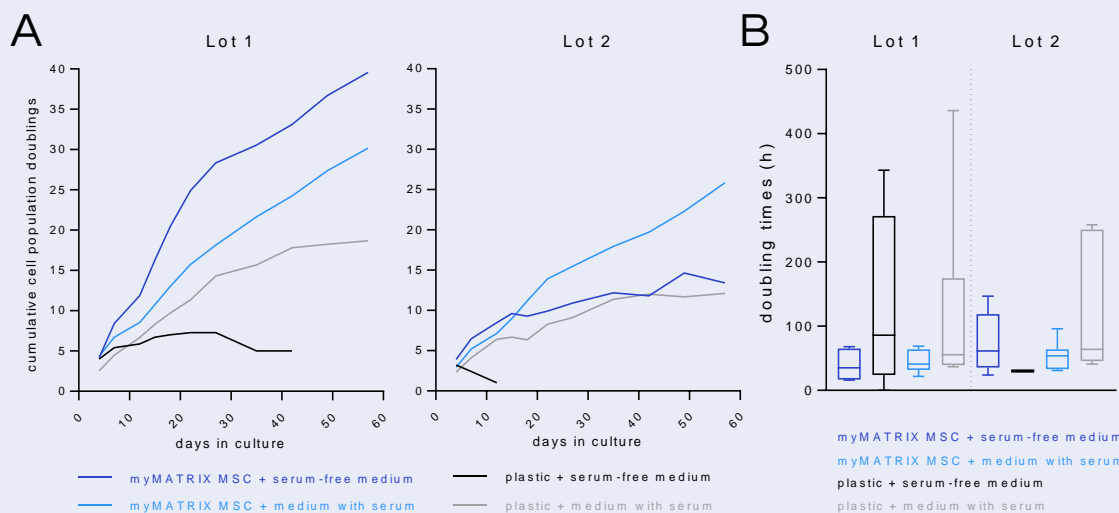
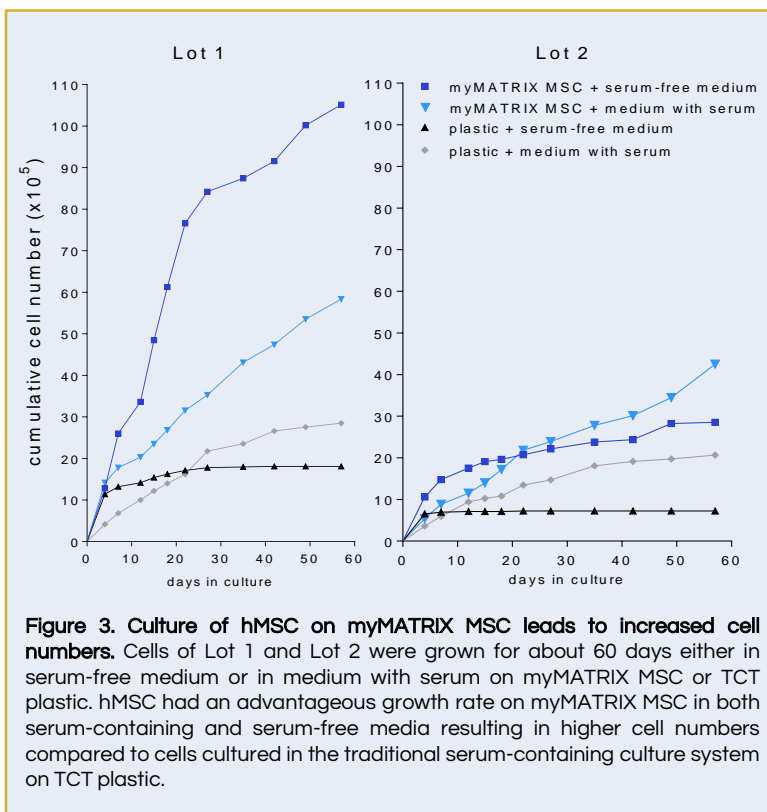


Table 3. Doubling time (h) minimum, median and maximum values for the indicated surface + media.

condition	Lot 1				Lot 2			
	myMATRIX MSC serum-free medium	myMATRIX MSC medium with serum	Plastic serum-free medium	Plastic medium with serum	myMATRIX MSC serum-free medium	myMATRIX MSC medium with serum	Plastic serum-free medium	Plastic medium with serum
Min	16	22	0	37	24	31	(30)	41
Median	35	41	86	56	62	54	(30)	64
Max	68	69	343	436	147	96	(30)	258

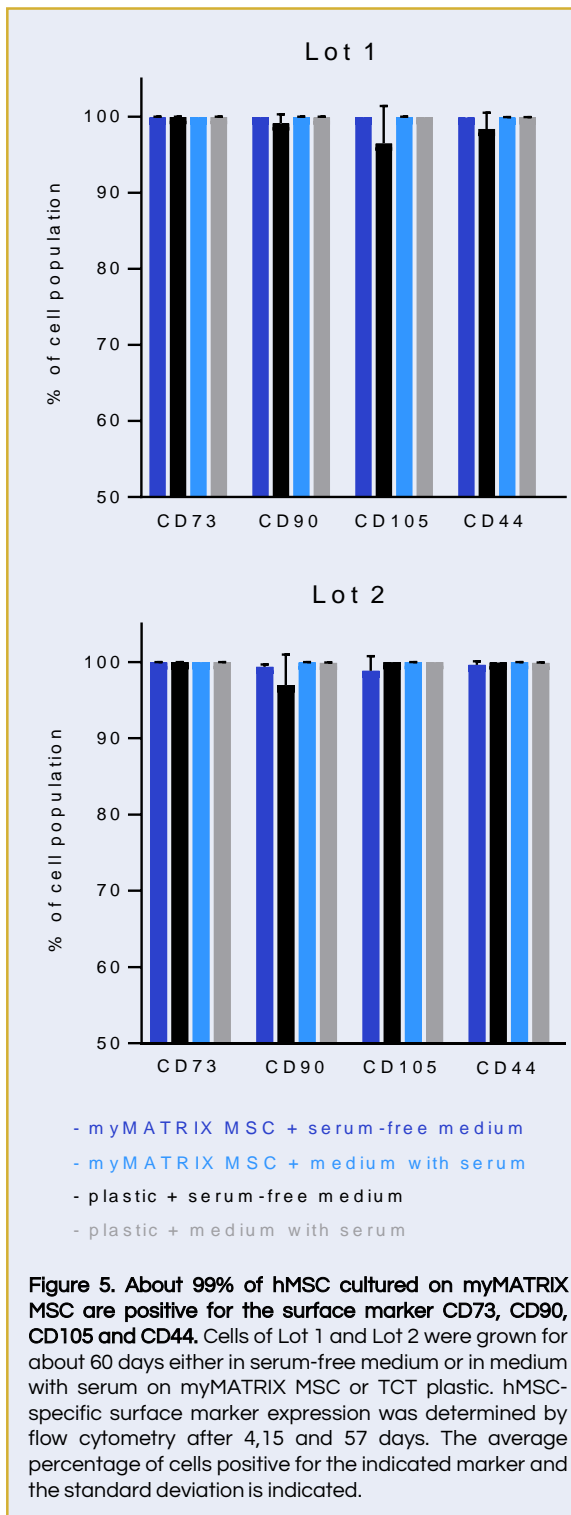
**hMSC retain surface antigens after long-term culture on myMATRIX MSC**

One minimal criterion for *in vitro* cultures of MSC is the expression of surface markers CD73, CD105 and CD90 in at least 95% of the cell population as defined by the International Society for Cellular Therapy. During the entire study, hMSC of both lots cultured on myMATRIX MSC in serum-free as well as serum-containing medium showed a typical expression profile of characteristic surface markers as evaluated by flow cytometry analysis (Fig. 5, table 4).

More than 95% of cells of the total cell population expressed the mesenchymal markers CD73, CD90 and CD105. In addition, about 99% of hMSC expanded on myMATRIX MSC maintained the expression of CD44, another surface marker highly expressed by hMSC. The marker expression profile obtained on myMATRIX MSC is comparable to the traditional serum-containing culture system on TCT plastic.

Table 4. Percentage of cell population of indicated condition positive for the indicated marker.

Lot	Culture surface	Culture system	CD73	CD90	CD105	CD44
1	myMATRIX MSC	serum-free	99.9	99.9	99.9	99.9
		with serum	99.9	100	99.9	99.9
	plastic	serum-free	99.9	99.1	96.5	98.4
		with serum	100	99.9	99.9	99.9
2	myMATRIX MSC	serum-free	99.9	99.3	98.8	99.6
		serum-free	99.9	99.9	99.96	99.9
	plastic	with serum	99.9	96.9	99.9	99.9
		with serum	99.9	99.8	100	99.9

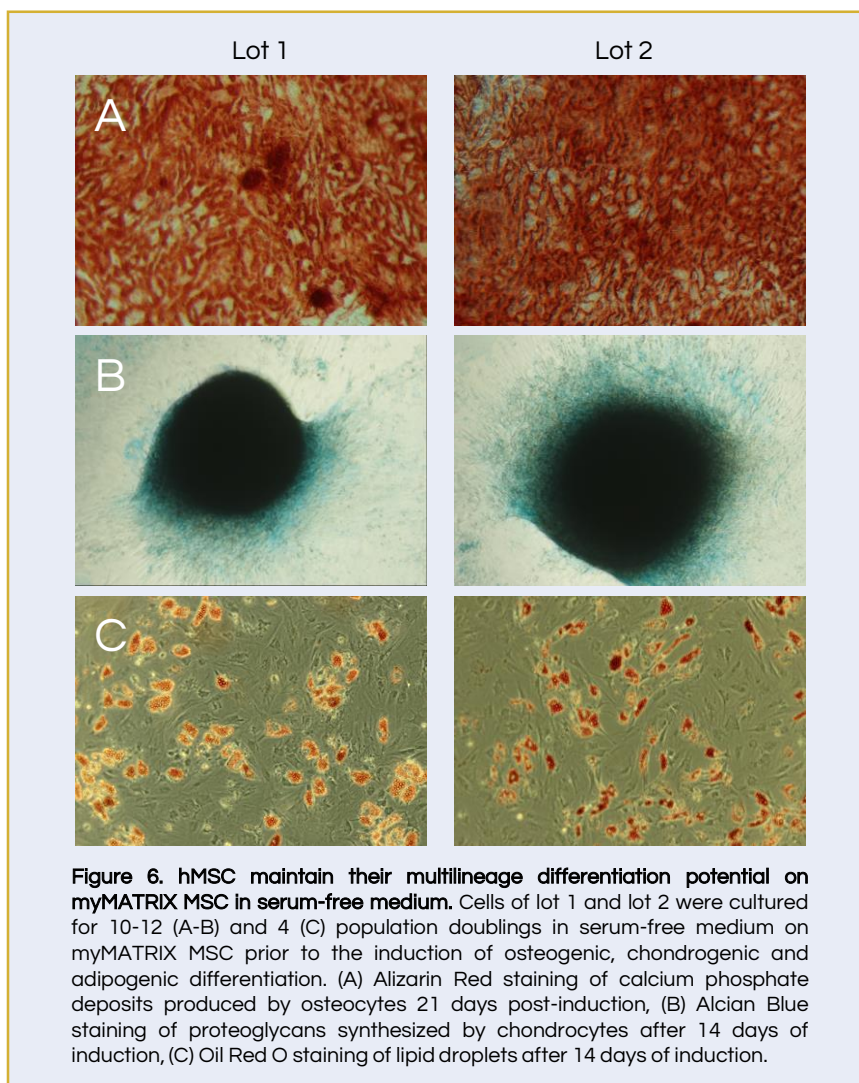




### ***hMSC cultured on myMATRIX MSC maintain multilineage potential***

To verify the multipotency of hMSC cultured on myMATRIX MSC in the presence (data not shown) or absence of serum (Fig. 6), different commercially available differentiation kits were used. Cells of both lots expanded for 4 population doublings in serum-free or serum-containing conditions were able to differentiate *in vitro* into the three specific mesoderm lineages: osteocytes, chondrocytes and adipocytes. Figure 6A shows stained mineralized deposits secreted by osteocytes differentiated from hMSC grown on myMATRIX MSC in serum-free conditions. In figure 6B, Alcian Blue staining of proteoglycans produced by hMSC-derived chondrocytes is displayed. Adipogenic differentiation of hMSC results in the accumulation of cytoplasmic lipid droplets visualized by Oil Red O staining (Fig. 6C).

In addition, cells expanded for 10-12 population doublings still showed high differentiation capacity for the osteogenic and chondrogenic lineage while the differentiation potential for adipocytes was reduced. Numerous reports have outlined that differentiation properties can vary due to different donors as well as distinct subtypes of hMSC, which can be predetermined by isolation or expansion media (8, 9). Moreover, cell age, shape and adhesion, seeding density and nutrient availability play key roles in efficient differentiation (10, 11). The impact of any of the aforementioned parameters on the adipogenic differentiation at high CPD needs to be investigated further. hMSC cultured in the traditional serum-containing culture system on TCT plastic were also able to differentiate into the three lineages (data not shown).

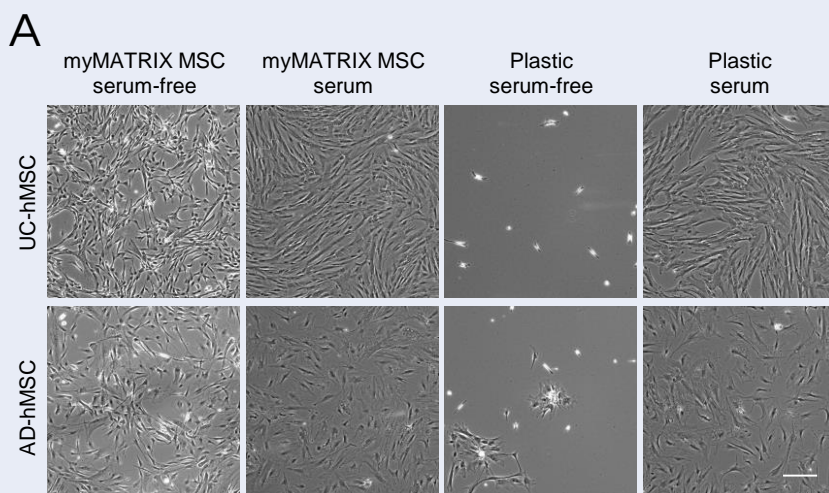


### *myMATRIX MSC supports long-term culture of adipose-derived and umbilical cord-derived hMSC*

Besides bone marrow (BM), hMSC have been isolated from other tissue sources including adipose tissue (AD) and umbilical cord (UC). Therefore, we examined the expansion of AD- and UC-hMSC for four serial passages in the four experimental conditions: 1) myMATRIX MSC/serum-free, 2) myMATRIX MSC/with serum, 3) plastic/serum-free, 4) plastic/with serum.

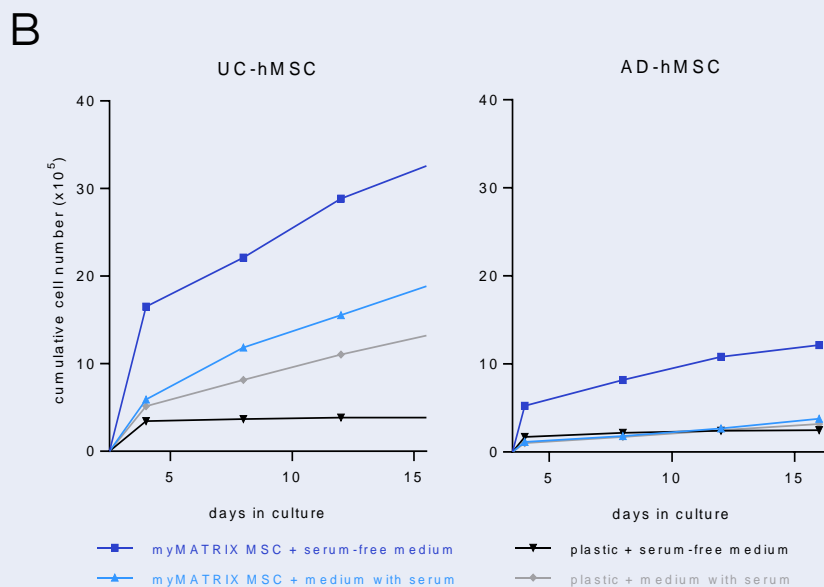
Similar to BM-hMSC, UC- and AD-derived cells maintained their characteristic fibroblast-like morphology (Fig. 7A) with AD-hMSC showing a more spread cell shape than UC-hMSC. Cells expanded in serum-free conditions acquired a spindle-shaped morphology as observed with BM-hMSC. In the absence of serum, only a small percentage of cells attached as single cells or small cell clusters on TCT plastic surface, with little to no spreading of the cells.

Cumulative cell numbers show that proliferation of UC- and AD-hMSC is enhanced on myMATRIX MSC, especially in serum-free medium (Fig. 7B, dark blue). Of note, although AD-hMSC of this donor show poor growth properties in serum-containing conditions a 3-fold higher cell number was observed on myMATRIX MSC in serum-free condition. In consistency with higher cell numbers, shorter doubling times were observed for cells cultured on myMATRIX MSC (data not shown). Cell viability was >90% for all experimental conditions except for cells grown in serum-free medium on TCT plastic. These results are consistent with the data obtained from BM-hMSC and show that myMATRIX MSC can support the long-term culture of hMSC from bone marrow, umbilical cord and adipose tissue.



**Figure 7. myMATRIX MSC supports enhanced proliferation of AD- and UC-hMSC in serum-containing and serum-free medium.**

(A) Representative cell morphology micrographs (day 12; phase contrast, 4x) for the indicated surface/media conditions are shown. UC- and AD-hMSC cultured in serum-containing medium display a fibroblast-like morphology on myMATRIX MSC as well as TCT plastic. AD-hMSC develop a more spread cell shape than UC-hMSC. myMATRIX MSC also supports attachment and growth in serum-free conditions with a more compact, spindle-shaped morphology. Due to the absence of attachment molecules in serum-free medium in combination with TCT plastic culturware only few cells loosely attach in this condition. Scale bar indicates 250  $\mu\text{m}$ . (B) UC- and AD-hMSC were grown for 16 days either in serum-free medium or in medium with serum on myMATRIX MSC or TCT plastic. The enhanced cell proliferation is demonstrated by higher cumulative cell number doubling on myMATRIX MSC compared to TCT plastic surfaces. hMSC in serum-free conditions on TCT were not able to attach and grow properly.



## Conclusion

The ready-to-use myMATRIX MSC supports the long-term expansion of hMSC in serum-containing as well as serum-free culture conditions. hMSC expanded on myMATRIX MSC show enhanced proliferation and high viability while maintaining their characteristic morphology, typical marker expression profile and multilineage mesodermal differentiation potential. Thus the recreation of essential elements of the cell microenvironment by myMATRIX MSC results in optimal growth conditions for hMSC.

In addition, myMATRIX MSC is ready-to-use thereby eliminating elaborate coating, washing steps or dedicated solutions. This not only reduces the risk of contamination, but also preparation time and effort while enhancing lot-to-lot consistency as well as reliable performance in comparison with self-coating solutions.

## Literature

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