PromoCell®

Xeno-free MSC culture: high yield, better viability and morphology

Application Note

Background

Human mesenchymal stem cells (hMSCs) have potential for massively impacting the fields of cell-based therapy, tissue engineering and regenerative medicine due to their ready availability, stem cell properties and immunomodulatory activity. In order to create optimal growth conditions for human mesenchymal stromal cells *in vitro*, it is essential to mimic specific aspects of the *in vivo* microenvironment. Cell culture media are often supplemented with fetal bovine serum to provide nutrients and growth factors that promote the survival and growth of cells. However, serum use also suffers from a number of disadvantages such as unknown composition, batch-to-batch variation, the risk of infectious agents and contaminants, and animal welfare concerns. Due to the physiological variation 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 regarding clinical applications.

Serum-free culture medium is an excellent alternative that enables controlled and reproducible conditions. Being adherent cells, hMSCs require attachment molecules on the culture surface, some of which are provided by the serum and consequently missing under serum-free conditions. hMSCs therefore often exhibit altered adhesion, morphology, and proliferation when cultured in serum-free media. To avoid this, media suppliers often recommend precoating of the cell culture plasticware with extracellular matrix protein extracts such as fibronectin. Another recently developed strategy enables researchers working with serum-free media to use a chemically defined surface coating that comes in a ready-to-use form. This innovative new product, called myMATRIX MSC, creates optimal growth conditions for hMSCs by using biomimetic peptides and complex oligosaccharide molecules to mimic the human body's extracellular matrix. myMATRIX MSC is suitable for scientists who don't want to use products of animal origin. At the same time, it ensures consistently good quality without any fluctuations. myMATRIX MSC delivers fast results and is both easy to handle and cost-effective.

Coating	Medium	Passage 0 (6)		Passage 1 (7)		Passage 2 (8)	
		Cell Count	Viability	Cell count	Viability	Cell Count	Viability
myMatrix MSC	MSC Growth Medium XF	5.7	99	5.6	99	4.6	99
	Serum-free competitor medium	4.8	97	5.3	97	4.6	93
Fibronectin (human)	MSC Growth Medium XF	3.7	96	4.15	97	2.8	98
Tissue culture treated plastic	Serum-comtaining	2.2	96	1.25	94	1.25	94
	MSC Growth Medium XF	2.0	94	1.8	97	2.05	95
	Serum-free competitor medium	1.2	91	0.97	85	0.66	85

Tab. 1: Cell counts (in x10^5) and viability (in %) of MSCs in passages 6, 7 and 8 on different coatings and with different media compositions. *Cells grown on myMATRIX MSC in serum-free media (PromoCell MSC Growth Medium XF, serum-free competitor medium) show very high cell counts and viabilities. The best performance was observed using a combination of myMATRIX MSC and our MSC Growth Medium XF, yielding very high cell counts and 99% viability throughout the study. Cell expansion on fibronectin-coated plates and our xeno-free medium resulted in lower counts and viabilities compared to myMATRIX MSC and serum-free medium. Cell growth and viability was reduced on uncoated plasticware.*

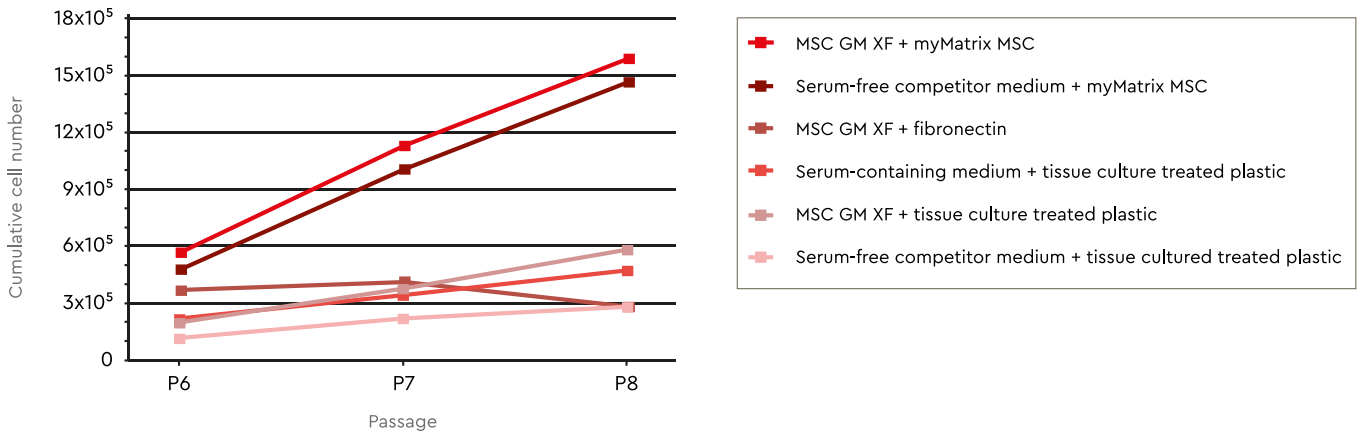

Fig. 1: Cumulative cell numbers of MSCs in passages 6, 7 and 8 on different coatings and with different media compositions. *The cumulative cell numbers demonstrate enhanced cell proliferation on myMATRIX MSC in serum-free medium, especially when using our MSC GM XF.*

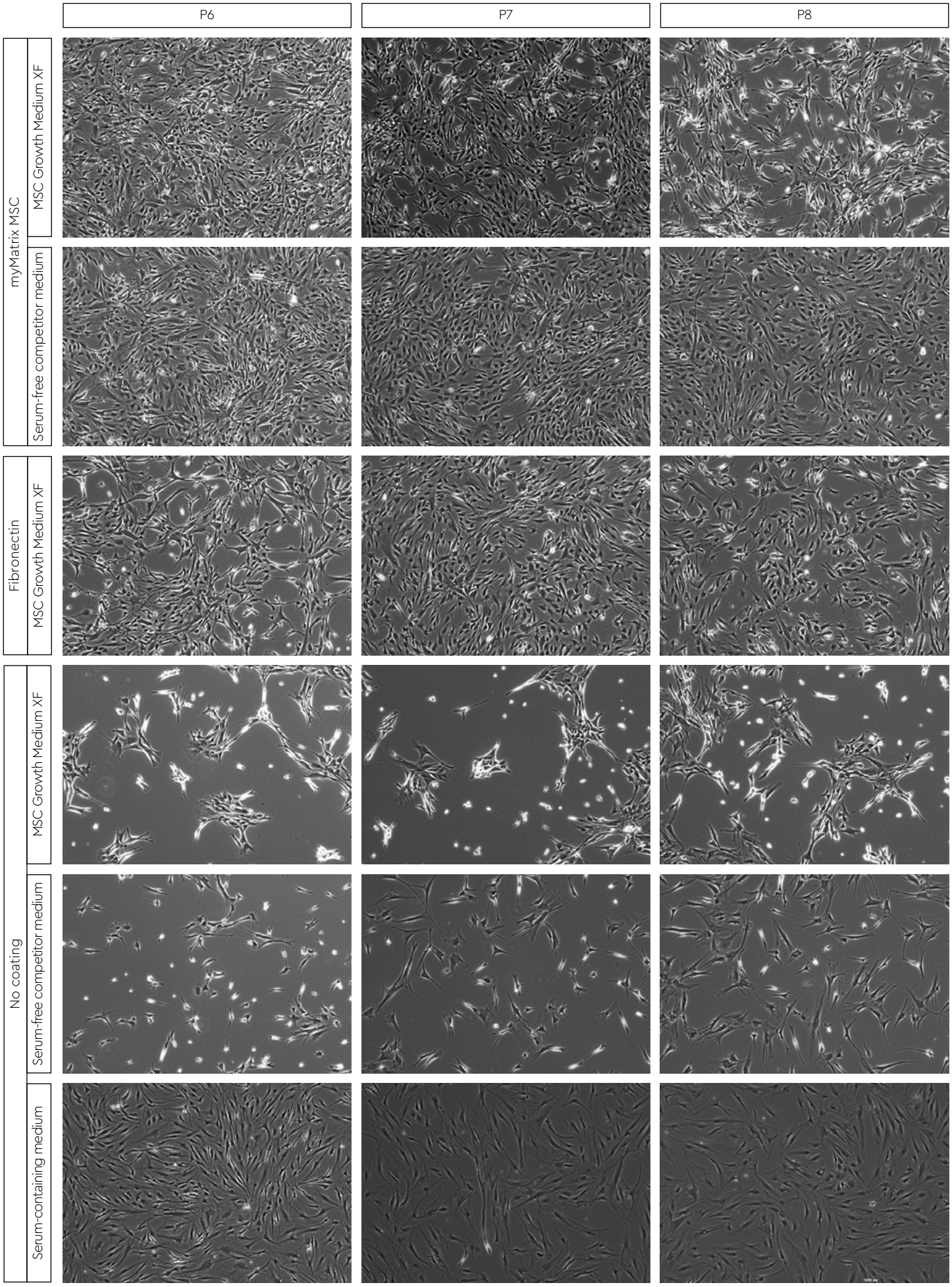

Fig. 2: Phase contrast images of MSCs in passages 6, 7 and 8 on different coatings and with different media compositions. *Cells grown in serum-free media (PromoCell MSC Growth Medium XF, serum-free competitor medium) on myMatrix MSC and on fibronectin-coated plates show a spindle-shaped, fibroblast-like morphology as well as smaller sizes than in serum-containing conditions. Tissue culture treated plasticware without coating was not sufficient to support MSC growth.*

Protocol for xeno-free expansion of MSCs

myMATRIX MSC are precoated plates that imitate the human extracellular matrix, thus providing optimal growth conditions for our human mesenchymal stem cells. Different media were used to compare cell culture conditions. MSCs were expanded until passage five in serum-containing medium on tissue culture treated plastic.

I. Serum- and xeno-free culture of MSCs on myMATRIX MSC

Materials

- Human bone marrow MSCs, passage 6 (C-12974)
- Serum-free medium: Mesenchymal Stem Cell Growth Medium XF (C-28019)
- Serum-free competitor medium
- Serum-containing medium: DMEM (low glucose, GlutaMAX™ Supplement, pyruvate, ThermoFisher Scientific, #21885025) + fetal bovine serum (10%, Sigma Aldrich, #F7524)
- Penicillin/Streptomycin (100 U/ml)
- Fibronectin from human plasma (Sigma Aldrich, #F2006)
- TrypLE Express (ThermoFisher Scientific, #12604013)
- Phosphate-buffered saline (PBS)
- Tissue culture treated cell culture vessels
  myMATRIX MSC (denovoMATRIX, #C0701)
  Trypan Blue stain

Use aseptic techniques and a laminar flow bench.

1

Fibronectin coating

Incubate the cell culture vessels with 5 µg/ml in PBS for two hours at 37°C.

2

Harvest and plate the cells

Harvest cells from existing culture at 70–80% confluence. Plate the cells at a density of 5.000 cells/cm² (5 ml per T25 flask) and incubate at 37°C and 5% CO_2. After four days take phase contrast images (4x).

3

Cell subculture

At 80% confluency, remove the medium from the flask, wash with PBS, and incubate the cells with TrypLE Express for three minutes at 37°C while tapping the flask. Then, stop trypsin activity by adding prewarmed serum-containing medium and gently rinse the flask. Centrifuge the cell suspension for three minutes at 210 x g, remove supernatant, and resuspend the pellet in corresponding growth medium. Afterwards, count the cells and determine the viability with an automated cell counter by using an aliquot of resuspended cell pellet with Trypan Blue stain (1:1)

4

Expand the cells

After three passages, seed the cells on a 24-well plate and let them grow for three days for fluorescent staining.

II. Fluorescent staining of MSCs

Materials

- 4% paraformaldehyde (PFA)
- Phosphate-buffered saline (PBS)
- PBS with Ca^{2+}/Mg^{2+}
- 0.1% TritonX solution
- Blocking buffer/antibody solution (3% donkey serum in PBS)

Use aseptic techniques and a laminar flow bench.

1

Wash and fix the cells

Wash the cells gently and briefly in PBS with Ca^{2+}/Mg^{2+}. Then, fix the cells with 4% PFA for 10 minutes and rinse them twice with PBS.

2

Permeabilize the cells

Permeabilize the cells with 0.1% TritonX for 15 minutes and rinse them three times with PBS.

3

Incubate the cells with blocking buffer for one hour

4

Primary Antibody

Incubate the cells with the primary antibody (Integrin β1/CD29, 1:500) solution for 1 hour. Then, wash the cells three times with PBS.

5

Secondary Antibody

Incubate with the secondary antibody (AlexaFluor 488, 1:500) solution for 30–60 minutes in the dark. Wash the cells afterwards four times with PBS.

6

Incubate the cells with phalloidin-Atto633

7

Incubate the cells with Hoechst solution for 10 minutes in the dark

Afterwards, wash the cells three times with PBS and directly proceed with imaging using BioTek Lionheart FX microscope 4x objective.

Results

- Proliferation: best performance on our MSC Growth Medium XF (PC MSC GM XF) + myMatrix MSC
- Viability: highest on PC MSC GM XF + myMATRIX MSC (99%)
- Morphology: spindle-shaped, fibroblast-like, smaller than in serum-containing condition
- Actin/integrin β1: prominent stress fibers in serum-free competitor medium myMATRIX/TCT and PC MSC GM XF/TCT, discrete integrin spots (focal adhesions) especially in PC MSC GM XF FN/TCT, integrin staining of myMATRIX PC MSC GM XF and serum-free competitor medium comparable

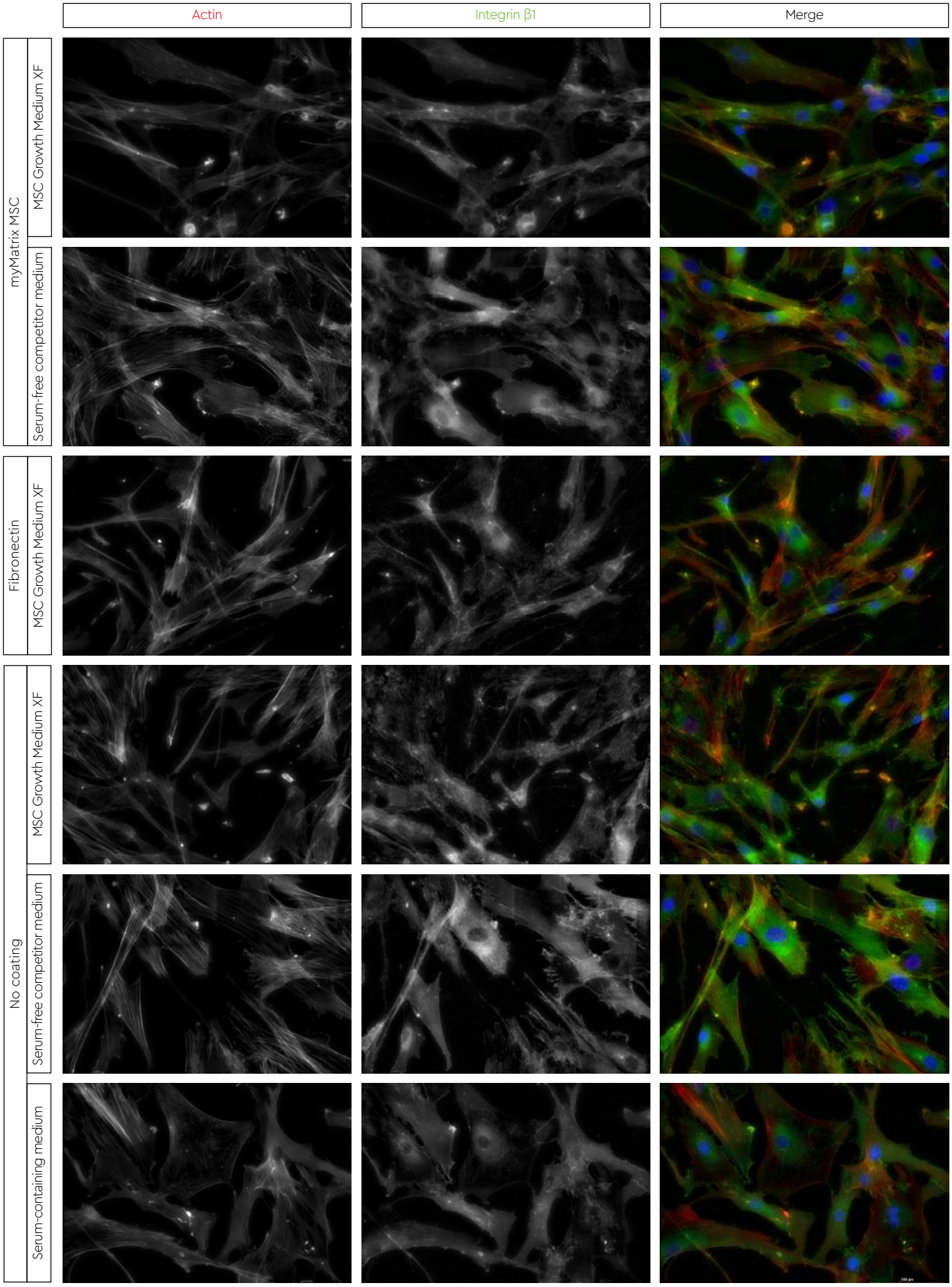

Fig. 3: Fluorescent stainings of MSCs in passages 6, 7 and 8. *Actin stress fibers are most prominent in cells cultured in PromoCell MSC Growth Medium XF and serum-free competitor medium without any coating. Discrete integrin spots (focal adhesions) are observed in all conditions, especially with our MSC Growth Medium XF on fibronectin-coated plasticware and without coating. The integrin pattern of cells grown on myMatrix MSC in serum-free media (PromoCell MSC GM XF and serum-free competitor) was comparable.*

Products

Product	Size	Catalog Number
Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)	500,000 cryopreserved cells 500,000 proliferating cells	C-12974 C-12975
Mesenchymal Stem Cell Growth Medium XF (ready-to-use)	500 ml	C-28019
screenMatrix www.denovomatrix.com/products/screenmatrix	96-well plate	S1001
myMATRIX MSC www.denovomatrix.com/products/mymatrix-msc	T75 / T25 24-well plate / 6-well plate	C0601 / C0701 C0301 / C0501

Related Products

Product	Size	Catalog Number
Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12971 C-12972
Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)	500,000 cryopreserved cells 500,000 proliferating cells	C-12977 C-12978
Mesenchymal Stem Cell Growth Medium 2 (ready-to-use)	500 ml	C-28009
Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (ready-to-use)	100 ml	C-28016
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (ready-to- use)	100 ml	C-28012
Mesenchymal Stem Cell Chondrogenic Differentiation Medium w/o Inducers (ready-to-use)	100 ml	C-28014
Mesenchymal Stem Cell Osteogenic Differentiation Medium (ready-to-use)	100 ml	C-28013
Mesenchymal Stem Cell Neurogenic Differentiation Medium (ready-to-use)	100 ml	C-28015
Accutase-Solution, primary human cell culture tested	100 ml	C-41310
Dulbecco's PBS, without Ca^{2+}/Mg^{2+}	500 ml	C-40232

This is a collaboration of:

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

07/2022