denovoMATRIX
ENABLING BIOLOGY IN VITRO

WHITE PAPER No. 472

myMATRIX iPSC – Xeno-free and chemically defined surface for high-quality pluripotent stem cell culture

Sandra Segeletz, Kristina Thamm, Michelle Patino, Akanksha Shukla, Thomas Hendel, Richard Wetzel and Dejan Husman

denovoMATRIX GmbH, Tatzberg 47, 01307 Dresden, Germany

Abstract

Human pluripotent stem cells (PSCs) are an attractive resource for cell therapy as they provide an unlimited supply of somatic cells. Their successful use requires a reproducible and cost-effective in vitro culture while maintaining essential cues of the natural cell environment. Specific matrices were developed in order to preserve the identity and integrity of PSCs but either are not suitable for cell therapy or lack to mimic the complexity of the natural extracellular matrix (ECM).

We have developed a chemically defined, animal and human component-free coating that recapitulates the function of the ECM by combining glycosaminoglycans with biofunctional peptides, called the myMATRIX iPSC. This matrix shows excellent single-cell attachment as well as robust and reliable growth in long-term culture. Importantly, PSCs grown on myMATRIX iPSC retain their pluripotency, genetic integrity and stemness character during long-term culture (approx. 140 days). Moreover, myMATRIX iPSC is a ready-to-use surface, which saves preparation time and effort while enhancing lot-to-lot consistency and reliable performance. These characteristics make myMATRIX iPSC excellently suitable for the development of animal source-free, high performance and reproducible cell culture protocols for stem cell research, drug development and cell therapy applications.

Introduction

Human pluripotent stem cells (hPSC) are self-renewing cells with the capability to differentiate into the three embryonic germ layers and hence into all somatic cell types. They can therefore be utilized for disease modelling, developmental biology as well as regenerative medicine and cell therapy. For the latter, induced pluripotent stem cells (iPSCs) are preferentially used over embryonic stem cells (ESCs) as they do not require the isolation from a developing embryo, which raises ethical concerns. iPSCs can theoretically be generated from any tissue by ectopic expression of four transcription factors (POU5F1 (also known as OCT4), SOX2, KLF4 and MYC) as shown by Yamanaka and colleagues (Takahashi & Yamanaka, 2006; Takahashi et al., 2007). Once generated, it is crucial that they remain faithful genetic copies of the cells they are derived from and that they retain their pluripotency during in vitro culturing. To ensure iPSC integrity and identity appropriate growth conditions have to be established. These growth conditions are mainly influenced by the medium and matrix composition used for culture. Medium is a major cost driver in cell culture and many formulations have been published with the attempt of developing more defined compositions

with reduced amounts of recombinant proteins to make significant savings (Ludwig et al., 2006; Chen et al., 2007; Yasuda et al., 2018; Kuo et al., 2019). Similar efforts were taken in the development of matrices supporting in vitro growth of PSCs. In vivo, the cells are supported by the extracellular matrix (ECM), which facilitates cell anchorage, serves as a growth factor reservoir and mediates biomechanical stiffness. The diverse functions of the ECM are realized by two classes of macromolecules, glycoproteins and glycosaminoglycans (GAGs). Plenty of matrices were developed as replacement of the ECM in vitro since PSCs are vulnerable to apoptosis (or anoikis) upon cellular detachment and dissociation (Gilmore, 2005). Originally, PSCs were cultured on mouse embryonic fibroblasts (MEFs) until the animal-derived growth substrate Matrigel proved to be a functional replacement as it consists of many ECM components such as laminin, collagen IV, heparin sulfate proteoglycans and nidogen. Matrigel is still widely used in the research community because of its proven functionality and established scientific record. Due to its animal origin and the possibility of batch-to-batch variabilities it is unsuitable for cell therapy. In order to culture iPSCs for cell therapy, many research groups have developed protein- or peptide-based growth substrates (Celiz et al., 2014) such as laminin-511 (Rodin et al., 2010), laminin-521 (Rodin et al., 2014), vitronectin (Braam et al., 2008), Synthemax™ II (Melkoumian et al., 2010) and cyclic RGD (Lambshead et al., 2018). Those matrices have proven to support PSC culture and partly subsequent differentiation but lack the complexity of the natural ECM (Burridge et al., 2014).

We have developed a synthetic, chemically defined, animal as well as human component-free coating for the culture of iPSC, which recapitulates the function of the natural ECM. It combines biologically relevant synthetic peptides with sulfated glycosaminoglycan analogs to facilitate iPSC adhesion, expansion and maintenance (Wieduwild et al., 2018). The incorporation of GAGs is beneficial for the adhesion-dependent and growth factor-sensitive iPSCs, since they support the stabilization of ligand-receptor interactions and storage of growth factors. This coating is ready-to-use and therefore saves preparation time and effort, while enhancing lot-to-lot consistency and reliable performance.

Material and Methods

Maintenance culture of hiPSCs

Human iPSCs (hiPSCs) (1: CRTD5, 2: 802-3G (REPROCELL), 3: CRTD4) were cultured in mTeSR™1 (STEMCELL Technologies) on different surfaces at 37°C and 5% CO_2 in a humidified incubator. The cells were monitored daily for morphological abnormalities, contamination and confluency using a phase contrast microscope. Large areas of differentiation were manually scraped-off using a glass pipette under the microscope. For passaging, hiPS cell colonies were dissociated using ReLeSR™ (STEMCELL Technologies) for 3-5 min following the manufacturer's protocol. This was followed by tapping the cultureware, flushing the cultures with a pipette and breaking the cell clumps into smaller pieces. The passaging was done at different split ratios depending on the surface and cell line.

Single-cell attachment, Cloning efficiency and Low cloning efficiency

When hiPSC culture reached 70-80% confluency, cells were dissociated using Accutase® (STEMCELL Technologies) to obtain single cells. The singularization was monitored under a microscope using a hemocytometer and the Accutase® reaction was stopped using 1 mL mTeSR™1. The dissociated cells were centrifuged at 250xg for 3 min and the pellet was resuspended in 1 mL mTeSR™1. The number of viable cells was counted using trypan blue staining. To determine the single-cell attachment efficiency, the hiPSCs were seeded at 40.000 cells/cm^2, 20.000 cells/cm^2 and 5.000 cells/cm^2. After the different time points (96 hours and 24 hours), non-adherent cells were carefully removed by rinsing the plates once with pre-warmed Dulbecco's modified Eagle medium (DMEM)/F12 (Gibco). The cells were fixed overnight at 4°C in 4% paraformaldehyde (PFA, w/v), washed twice with phosphate buffered saline (PBS) and subsequently stained with crystal violet. To quantify the attachment of single-cells after 24 hours of culture, the percentage of surface area fraction covered by positively stained hiPSCs was calculated using Fiji software. The 96-hour time points were quantified by measuring the absorbance of the crystal violet release.

Quantitative polymerase chain reaction (qPCR)

At passage 10, hiPSCs from one 6-well were washed, detached with ReLeSR™ as described before and then pelleted, washed again and frozen until further processing. The RNA isolation and qPCR were performed by a service partner.

Fluorescence-activated cell sorting (FACS)

Flow cytometry analysis of pluripotency markers was performed using the LSR II flow cytometer at the Stem Cell Engineering Facility at CMCB Technology Platform (TU Dresden). Human iPS cells were detached using TrypLE Express Enzyme (Thermo Fisher Scientific), washed and 200.000 cells per sample were fixed with 1% PFA for 10 min at room temperature (RT), washed and permeabilized with 0.01% Triton X-100 for 5 min at RT. Cells were then stained with Alexa Fluor 488 Mouse anti-Oct3/4 (560253), Alexa Fluor 647 Mouse anti-human TRA-1-60 Antigen (560122), PE Mouse anti-Sox 2 (560291) and V450 Mouse anti-SSEA-4 (561156), all from BD Biosciences BD FACSDiva and FlowJo software were used for analysis.

Karyotyping

Cytogenetic analysis using G-banding was performed with a minimum of 350 (at P10-15) or 400 bands (at P20) for all samples. A minimum of 25 metaphases were analyzed numerically and at least 5 metaphases were analyzed structurally per probe. Karyotype and band resolution were set up according to ISCN 2016.

Tri-lineage differentiation

After long-term culture (P20), cells of each line expanded on the different surfaces were differentiated using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) according to the manufacturer's protocol on Matrigel-coated 24 well plates (µ-Plate 24 Well Black, ibidi, 82406). After the designated differentiation times, cells were fixed for 15 mins at 37°C with 4% PFA, washed multiple times with PBS before they were permeabilized with 0,1 % Triton X-100 and blocked with 3% BSA (w/v). Afterwards, cells were incubated with primary antibodies (anti-Nestin 1.25 µg/mL (BD Transduction laboratories, #611659); anti-Pax6 2-3 µg/mL (Invitrogen, #44-6600); anti-Brachyury 10 µg/mL (Invitrogen, #PA5-46984), anti-CXCR4 Clone 12G5 5-10 µg/mL (Stemcell technologies, #60089), anti-Sox17 4 µg/mL (Biolegend, #698502); anti-GATA6 10 µg/mL (Invitrogen, #PA1-104) overnight at 4°C, followed by the corresponding secondary antibodies and DAPI for 30 mins at RT.

Microscopy and statistical analysis

Images were taken using a Lionheart XF micro-

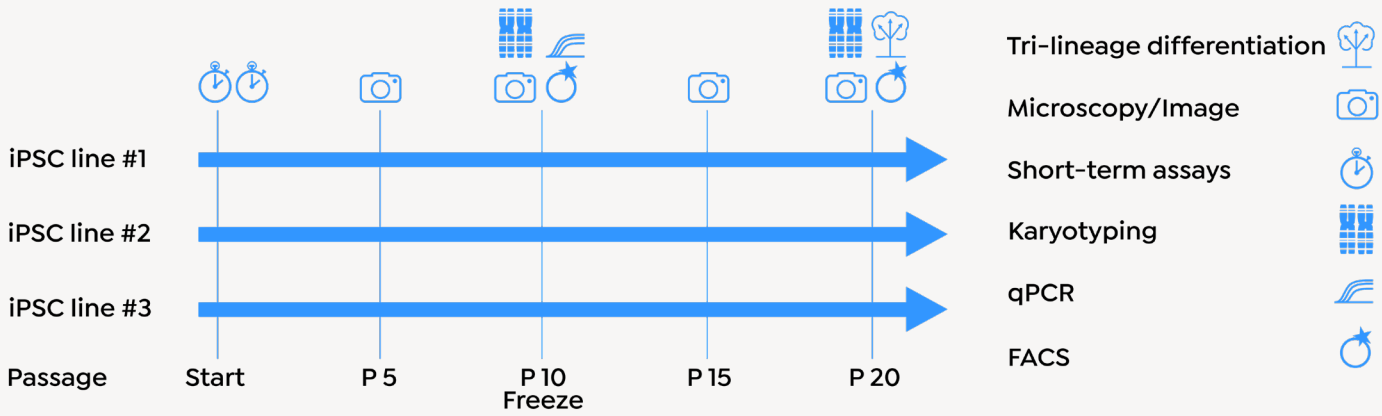

Figure 1. Experimental workflow of long-term culture. Three different iPSC lines were cultured for 20 passages. Experiments characterizing initial attachment and attachment at clonal seeding densities were performed at the beginning. Every 5 passages images of the cells were taken at confluency. At P10, all cells were characterized using FACS analysis, karyotyping and qPCR. Subsequently, iPSCs were frozen and stored in liquid nitrogen. Cells were reseeded from frozen stocks and characterized again using FACS and karyotyping at P20. In addition, cells were differentiated into the 3 embryonic germ layers.

scope (Biotek) equipped with a 16-bit CMOS camera and analyzed using the Fiji software. Statistics and data processing were carried out using the Graph Pad Prism software, version 9.0.1.

Results and discussion

Experimental setup of long-term culture

In order to evaluate the functionality of the myMATRIX iPSC and its ability to support long-term culture of human iPSCs (hiPSC) we sought out to culture cells over 20 passages. In addition to the long-term culture, the 3 hiPSC lines were used to characterize initial attachment of cells to the surface and attachment at clonal densities. Throughout the experiments we compared the performance of our surface with Matrigel, as it is still widely used for PSC culture. At the beginning of the long-term experiment, cells were thawed from frozen stocks and seeded into 6-wells with Rho-associated, coiled-coil containing protein kinase (ROCK) inhibitor at a density of 200.000 cells/well. Along the culture, cells were split as clumps using ReLeSR™ at a standardized ratio of 1:20-1:30 to ensure a manageable splitting routine in the 6-well format and different characterizing assays were performed to ensure iPSCs integrity (Figure 1). The morphology of the iPSCs was monitored by phase contrast microscopy and images were taken at confluency at passages 5 and 10. After 10 passages, approximately 80 days in culture, cells were analyzed for their expression of pluripotency-specific markers by quantitative PCR (qPCR) and fluorescence-activated cell sorting (FACS). In addition, they were checked for genetic integrity using karyotyping, frozen and stored in liquid nitrogen.
For the subsequent 10 passages cells were brought back into culture using ROCK inhibitor as described before. After recovery from thawing for one passage, cells were cultured and morphologically monitored as before. In passage 20, iPSCs

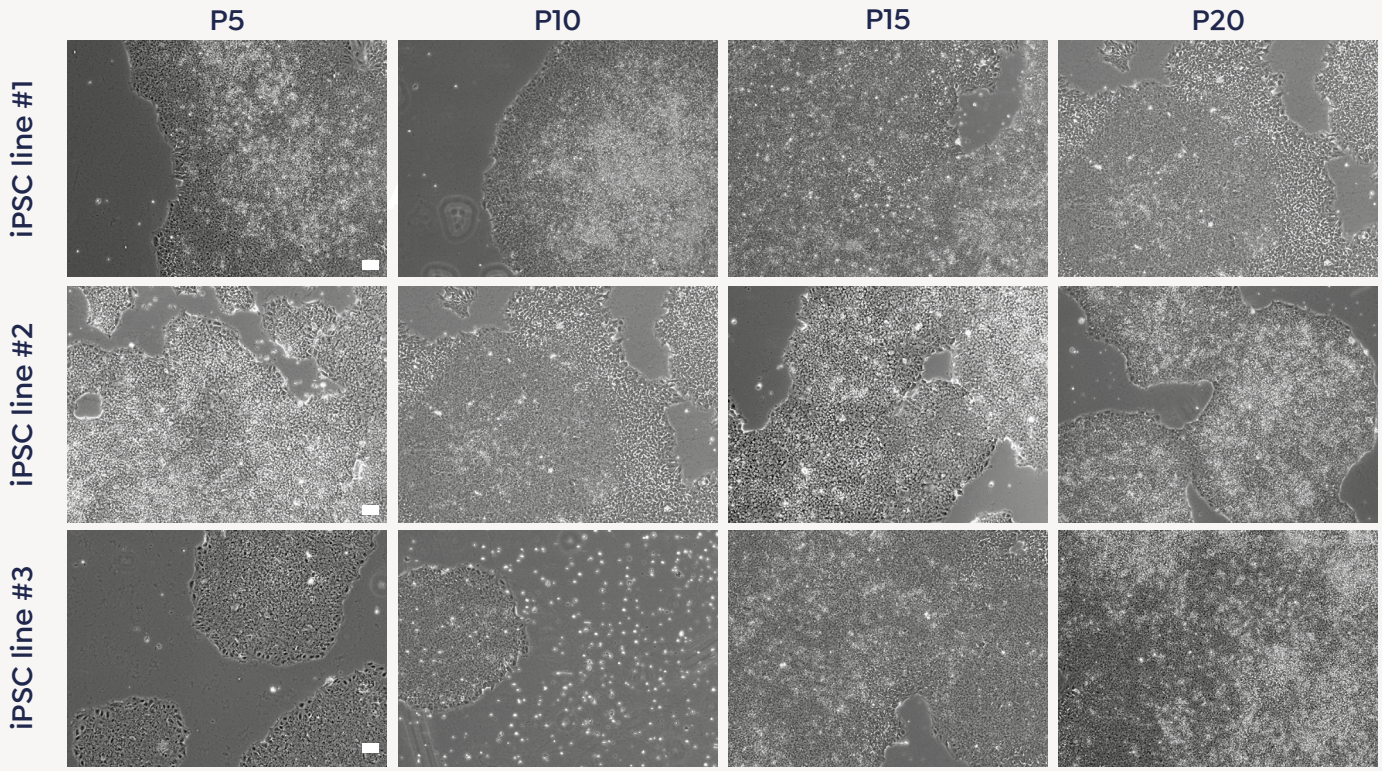

Figure 2. Representative images of hiPSC culture on myMATRIX iPSC. hiPSC lines grown on myMATRIX iPSC were imaged after every 5 passages in culture using phase contrast imaging. The cells show an iPSC-characteristic colony morphology with compacted individual cells that exhibit a high nuclear-to-cytoplasmic ratio. Scale bar: 100 µm

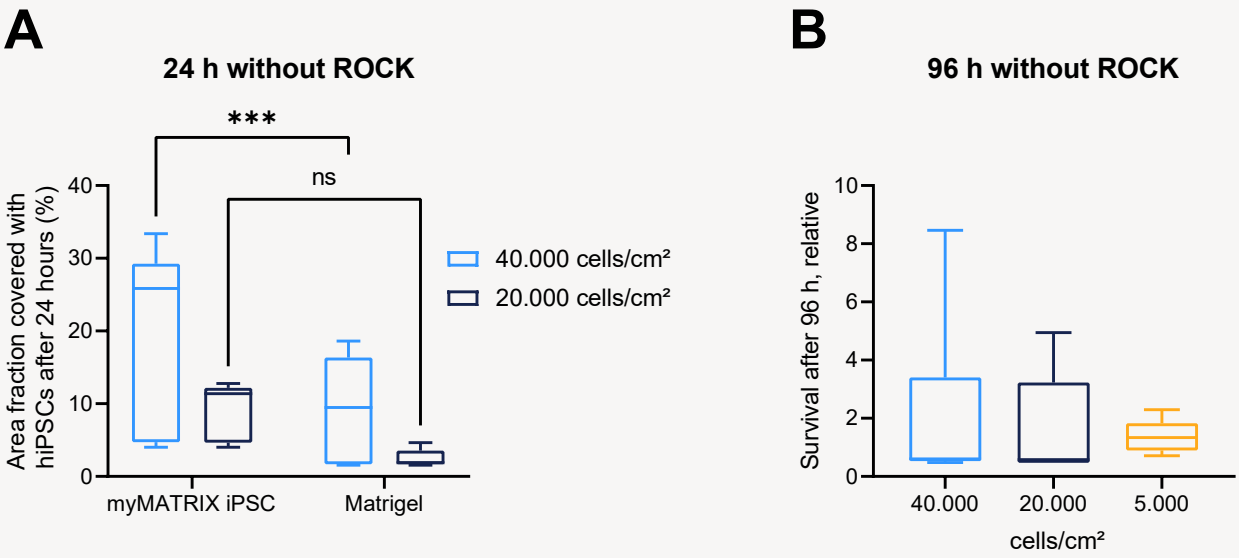

Figure 3. Initial attachment and survival of dissociated hiPSC. For the analysis of initial attachment and survival of dissociated hiPS cells on myMATRIX iPSC and Matrigel, cells were dissociated using Accutase and subsequently seeded onto the coated surfaces. After fixation, cells were stained with crystal violet and either area fraction or crystal violet release was measured. A) Survival of dissociated hiPSC after 24 hours on Matrigel and myMATRIX iPSC, B) survival of dissociated hiPSC after 96 hours of culture normalized to the number of cells on Matrigel. Error bars show standard deviation. Experiments were conducted with 5 technical replicates and 3 biological replicates. Statistical significance was calculated using Šídák's multiple comparisons test (*** $p<0.001$).

were again objected to a FACS analysis and karyotyping. In addition, cells were assessed for their pluripotency by tri-lineage differentiation.

hiPSC cultured on myMATRIX iPSC maintain their characteristic morphology

HiPSCs are small cells with distinctive round and compact morphological features, which are often used as an indicator of their undifferentiated state. During the long-term culture cells were checked daily for their morphology and signs of differentiation. Additionally, we recorded the morphology of the cells on the myMATRIX iPSC surface and Matrigel at confluency every 5 passages. Each of the iPSC lines used, showed slightly different morphologies, as in different compactness, on both Matrigel and myMATRIX iPSC. At confluency, hiPSCs grown on myMATRIX iPSC showed a similar morphology to Matrigel throughout all passages (Figure 2). After splitting however, cells on myMATRIX iPSC were less compact than on Matrigel (data not shown).

Single-cell iPSCs show strong attachment to myMATRIX iPSC in the absence of ROCK inhibitor

Poor clonogenicity is common for hPSC cells, most ly because they are prone to anoikis upon single-cell dissociation. At cell concentrations below 5.000 cells/cm², the survival of PSCs is rapidly reduced as they are not able to contact each other soon enough (Rodin et al., 2014). However, good attachment at clonal densities is important for certain applications such as genetic engineering, where clonal isolation and expansion is needed. Therefore, inhibitors of anoikis such as ROCK inhibitor are frequently used for clonal expansion but prolonged exposure to ROCK inhibitor has been implicated to adversely affect iPSCs (Watanabe et al., 2007; Vernardis et al., 2017; Gao et al., 2019). Here, we focused on initial attachment of iPSCs 24-hours post-seeding and their proliferation/survival af-

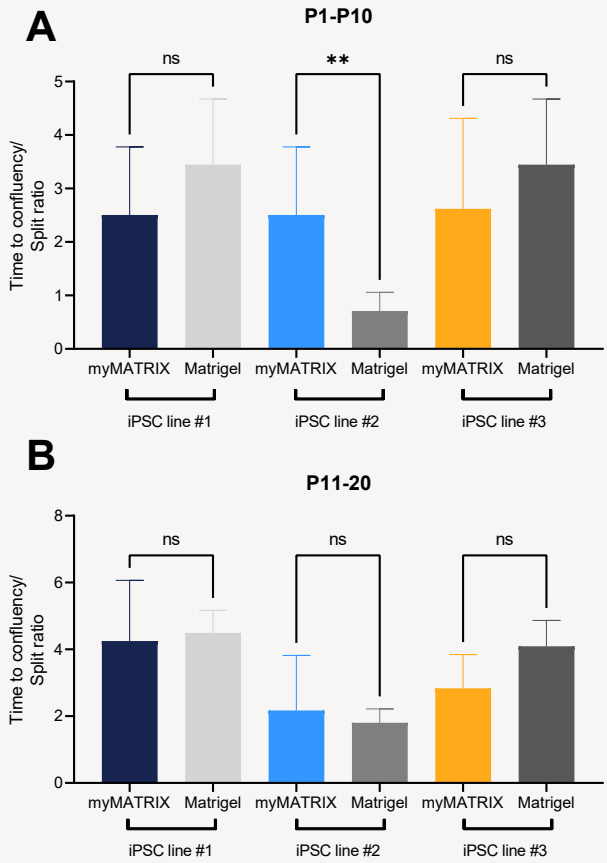

Figure 4. Growth speed of hiPSC during long-term culture. HiPSC were cultured on myMATRIX iPSC and Matrigel for 20 passages. Growth speed was calculated as a ratio of time until confluency was reached divided by the corresponding splitting ratio. The data is displayed separately for A) P1-10 and B) P11-20 as cells were frozen at P10. Mean±standard deviation is shown. Statistical significance was calculated using Šídák's multiple comparisons test (** p<0.01).

ter 96-hours of culture after single-cell dissociation in the absence of ROCK inhibitor. Note that we did not find any significant difference between Matrigel and myMATRIX iPSC when ROCK inhibitor was used. For the single-cell experiments, cells were dissociated with Accutase and seeded in different concentrations onto the wells coated with either Matrigel or myMATRIX iPSC. After 24 hours cells were fixed and stained with crystal violet and the area occupied by the cells was measured. At this time point, the 5.000 cells/cm^2 condition showed hardly cells, a proper measurement was therefore not possible. At a concentration of 40.000 cells/cm^2, myMATRIX iPSC displayed a significantly higher attachment of iPSCs than Matrigel (Figure 3A). Although higher, the difference was not significant at 20.000 cells/cm^2 (p=0.0665). After 96 hours, we found a 1.4-, 1.6- and 2-fold higher number of cells on myMATRIX iPSC compared to Matrigel for the 5.000, 20.000 and 40.000 cells/cm^2 condition respectively (Figure 3B). In some experiments we found up to 8-fold higher attachment of single cells to myMATRIX iPSC, however the response remained variable depending on the cell line. These results indicate that myMATRIX iPSC has a better initial cell attachment and survival than Matrigel when iPSCs are seeded as single cells. It is therefore better suitable for media or routines that employ single-cell splitting.

iPSCs on myMATRIX iPSC proliferate reliably and robust

The long-term culture (20 passages) of three iPSC lines was conducted for 140-160 days, depending on the line. In order to reflect their respective growth speeds, we defined a time-to-ratio quotient calculated by the time until cells reached confluency divided by the ratio at which cells were split. At the beginning, single cells were seeded and the assessment was done in two blocks of 10 passages each. Generally, the cells were proliferating reliably and robust throughout the 20 passages. We found no significant difference in growth speed of the cells cultured on Matrigel or myMATRIX iPSC for iPSC line #1 and #3 in the first 10 passages (Figure 4A) as well as the subsequent 10 Passages (Figure 4B). For iPSC line #2, multiple attempts were necessary for successful initial attachment of the cells to Matrigel with an adaptation phase of up to 20 days (data not shown). This problem did not occur on myMATRIX iPSC and cells therefore grew significantly faster (Figure 4A). Although the hiPSC of line #2 grew still faster on myMATRIX iPSC in the passages 11-20, the difference was not significant any more after the prolonged adaption of the cells to Matrigel. (Figure 4B). The adaptation problems of line #2 on Matrigel may be caused by its reprogramming

on a defined surface. In summary, our data shows that myMATRIX iPSC is a suitable alternative xeno-free surface with similar growth rates as Matrigel.

myMATRIX iPSC retains a high stemness in iPSCs

The analysis of the expression of several pluripotency-associated markers provides a useful tool to assess the ongoing long-term culture of iPSCs. At passage 10, we therefore measured the number of mRNA transcripts of the pluripotency markers OCT4, NANOG, Sox2, miR-302 HT, FGF2 and Lin28. The highest value per analyzed gene was used for normalization. Cells from the iPSC line #1 grown on myMATRIX iPSC showed a 2-fold increase in OCT4 expression and conversely a 2-fold lower expression of Sox2 (Fig. 5A). Lin28, miR-302 HT as well as Nanog showed a slightly lower or equal expression in myMATRIX iPSC compared to Matrigel. Interestingly, cells from iPSC line #2 showed an approximately 3-fold increase in FGF-2 expression whereas Sox2 was 2-fold decreased. The other genes were expressed similarly in Matrigel and myMATRIX iPSC. The strongest expression of stemness-related genes was found in iPSC line #3 grown on myMATRIX iPSC, where especially OCT4 was highly expressed (2-3-fold higher than on Matrigel). In sum, cells grown on myMATRIX iPSC displayed a higher expression of stemness markers compared to Matrigel as visualized in the pile-up view (Fig. 5B) suggesting that myMATRIX iPSC supports the maintenance of stemness at a very high level.

iPSCs grown on myMATRIX iPSC show strong expression of pluripotency-associated genes

The characterization of antigen expression by FACS using a specific set of markers provides useful information on cell state and the number of differentiated cells in culture. These are key from a safety perspective as spontaneous differentiation of cells poses a risk for the patients during clinical trials. The immunophenotyping also indicates the variability in the expression of stemness markers throughout long-term culture. Therefore, hiPSC were stained with PSC specific intracellular markers OCT4 and Sox2 as well as with cell-surface markers SSEA-4 and Tra-160 and analyzed by FACS. The marker analysis confirmed the high expression levels of

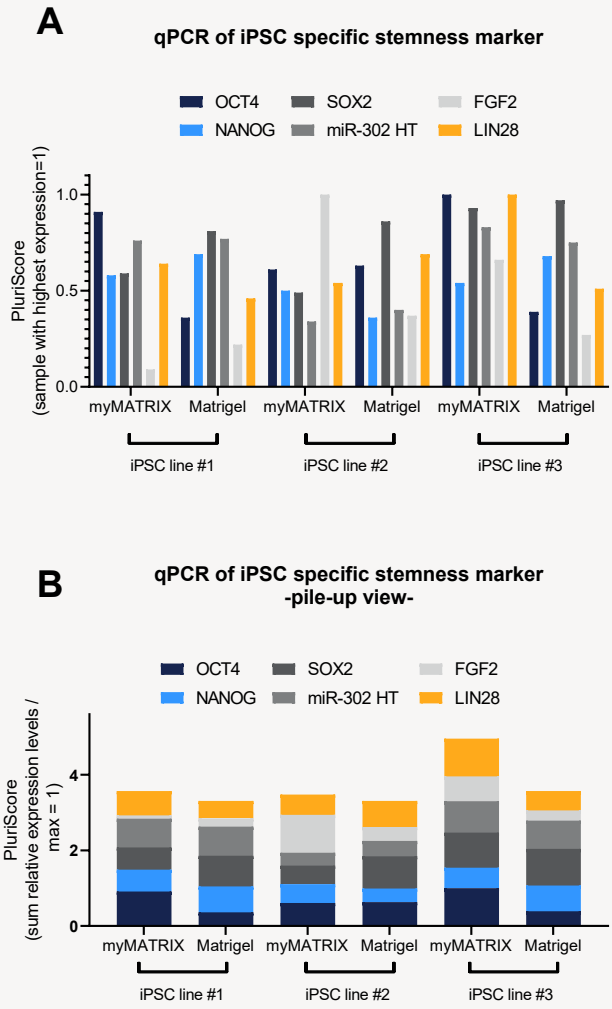

Figure 5. Quantitative qPCR of iPSCs cultured on myMATRIX iPSC and Matrigel for 10 passages. HiPSC at passage 10 were assessed for their expression of pluripotency-associated genes OCT4, Sox2, FGF2, Nanog, miR-302 HT and Lin28 using qPCR analysis. Data is expressed as Pluriscore, in which the sample with the highest expression was set to 1. A) Individual view of genes expressed in the sample and B) Pile-up view.

stemness markers as found using qPCR. Cells of line #1 grown on myMATRIX iPSC showed a superior expression of stemness markers at both time points of analysis (P10-14 and P20) compared to Matrigel (Figure 6A). About 96-100% of the cells of iPSC line #2 showed expression

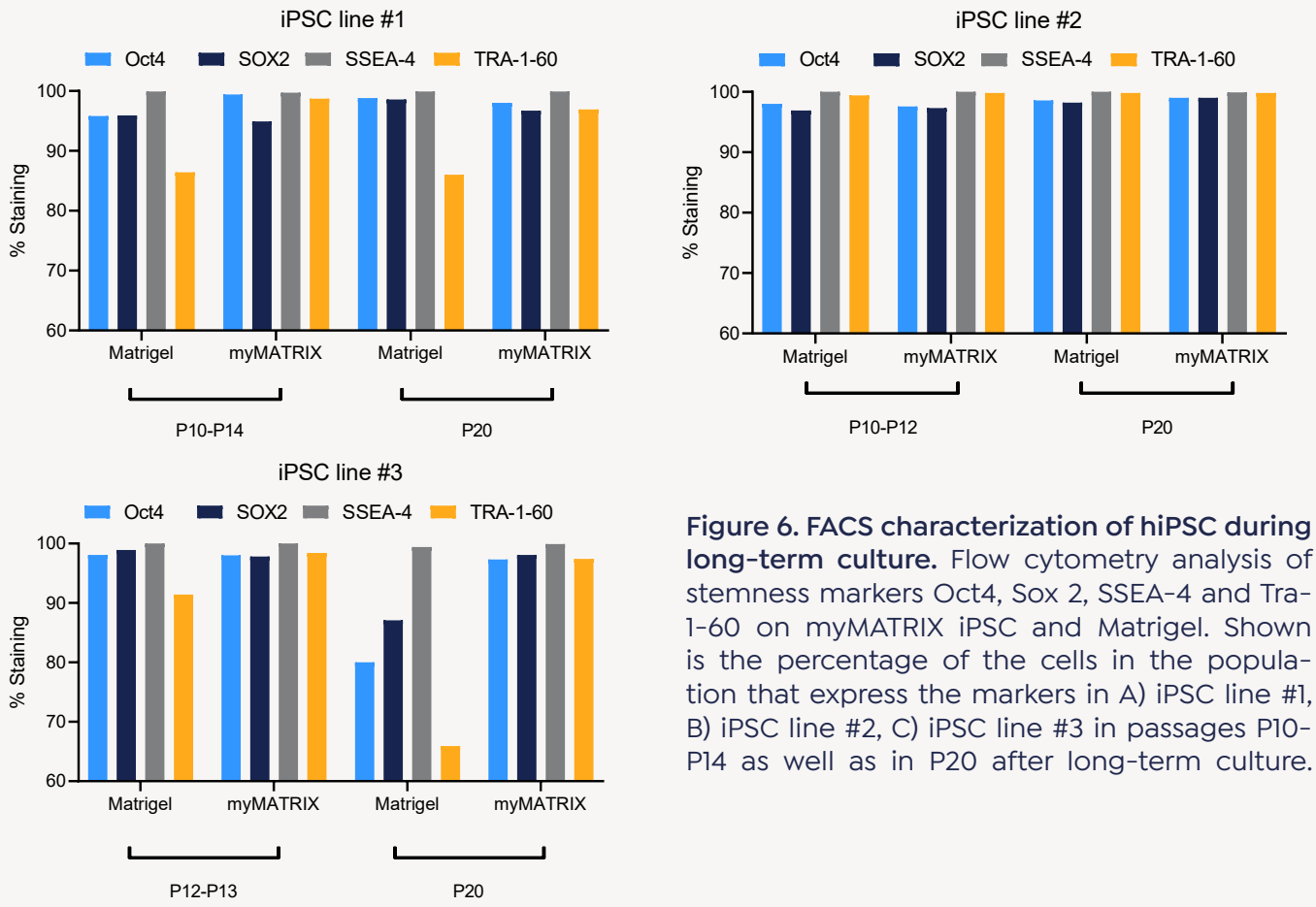

Figure 6. FACS characterization of hiPSC during long-term culture. Flow cytometry analysis of stemness markers Oct4, Sox 2, SSEA-4 and Tra-1-60 on myMATRIX iPSC and Matrigel. Shown is the percentage of the cells in the population that express the markers in A) iPSC line #1, B) iPSC line #2, C) iPSC line #3 in passages P10-P14 as well as in P20 after long-term culture.

of stemness markers throughout the culture on both Matrigel and myMATRIX iPSC (Figure 6B). Substantial differences in the percentage of positively stained cells between Matrigel and myMATRIX iPSC were found for iPSC line #3. The decrease in stemness marker expression on Matrigel resembled the high susceptibility of this line for spontaneous differentiation (Figure 6C). In contrast, myMATRIX iPSC was able to maintain hiPSC of high quality as > 95% of the cells did express all of the above-mentioned markers of the undifferentiated state

Long-term culture of iPSCs on myMATRIX iPSC does not compromise their genetic integrity
Genetic changes in iPSCs raise major concerns for the safety of iPSCs in clinical applications as transformation may lead to malignancy in patients (Tapia & Schöler, 2016). Especially iPSCs have been proposed to be susceptible to genetic changes in prolonged culture and genomic integrity has to be regularly checked (Peterson et al., 2011; Initiative, 2012). We performed cytogenetic analysis for all cell lines at P10 and P20 using G-banding at a band-level resolution of at least 200 (at P10-15) or 400 bands (at P20). The analysis of cultured iPSCs revealed a 46, XY or 46, XX normal male/female karyotype with no consistent numerical or structural abnormality in the cells (Figure 7).

myMATRIX iPSC maintains iPSCs pluripotency
As a functional test for the differentiation capacity of long-term cultivated hiPSC we differentiated the cells at P20 into all three embryonic germ layers using a single-cell split and a monolayer culture. As markers for ectoderm, we chose the intermediate filament protein Nestin and the transcription factor Pax 6. On day 7 after ectoderm induction

almost all of the cells expressed Nestin and Pax 6 as seen in Figure 8. The mesodermal markers Brachyury (transcription factor) and CXCR4 (receptor) were both uniformly expressed in the differentiated cells at day 4 after induction. For the assessment of differentiation efficiency into the endodermal lineage we stained the iPSCs for transcription factors Sox17 and GATA6 4 days after induction. Cells grown on myMATRIX iPSC for 20 passages were also able to efficiently differentiate into endoderm. The iPSCs grown on Matrigel underwent the same functional test. We could not find a qualitative difference between the differentiation capacities on Matrigel or myMATRIX iPSC. In summary, the iPSCs grown on myMATRIX iPSC highly express pluripotency-associated genes, remain karyotypically stable, proliferate stably over long-term culture and are able to differentiate into all germ layers.

Conclusion

In conclusion, myMATRIX iPSC is a powerful xeno-free alternative to Matrigel that reliable maintains the pluripotency of hiPSCs. Growth speed, attachment capacity, morphology, maintenance of stemness and differentiation capacity as well as genetic integrity are equal or superior to the widely used Matrigel. Moreover, using myMATRIX iPSC removes the need for tedious precoating procedures.

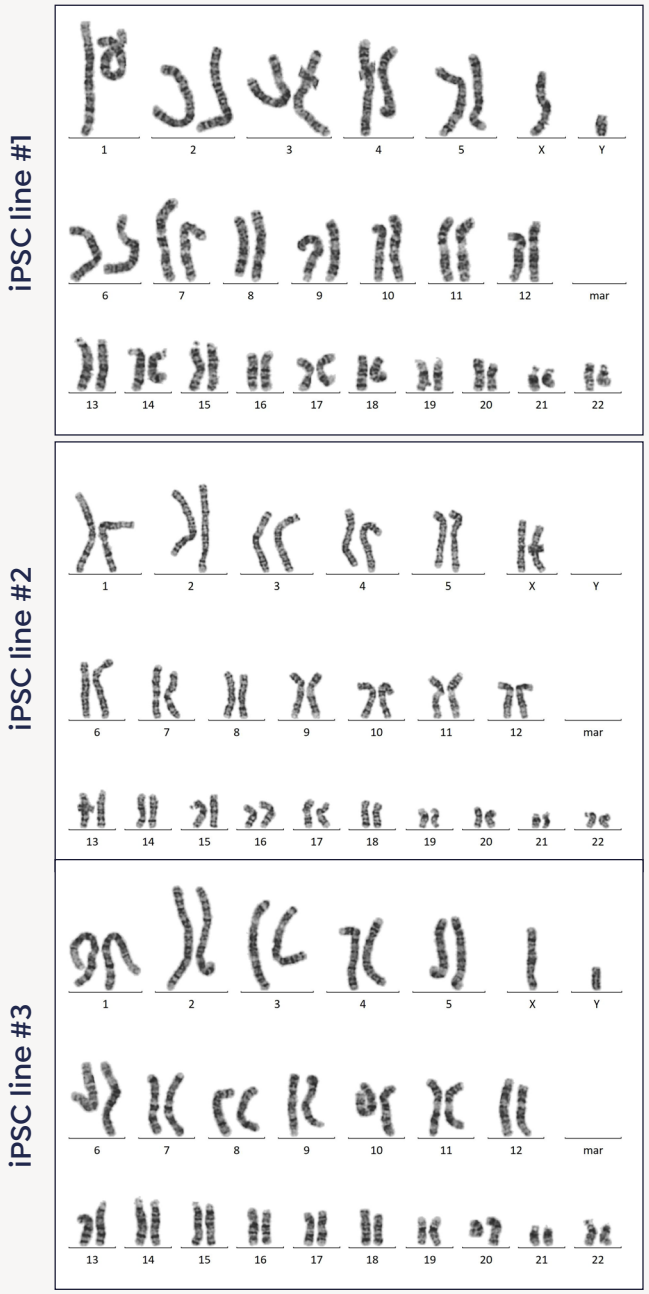

Figure 7. Karyotypic analysis. Shown are representative karyotypes of each cell line after long-term culture on myMATRIX iPSC at passage 20. Cytogenetic analysis using G-banding at a band level resolution below 400 revealed a 46, XY or 46, XX normal male/female karyotype at P10 and P20 for myMATRIX as well as for Matrigel. No consistent numerical or structural abnormalities were observed in the metaphases analysed.

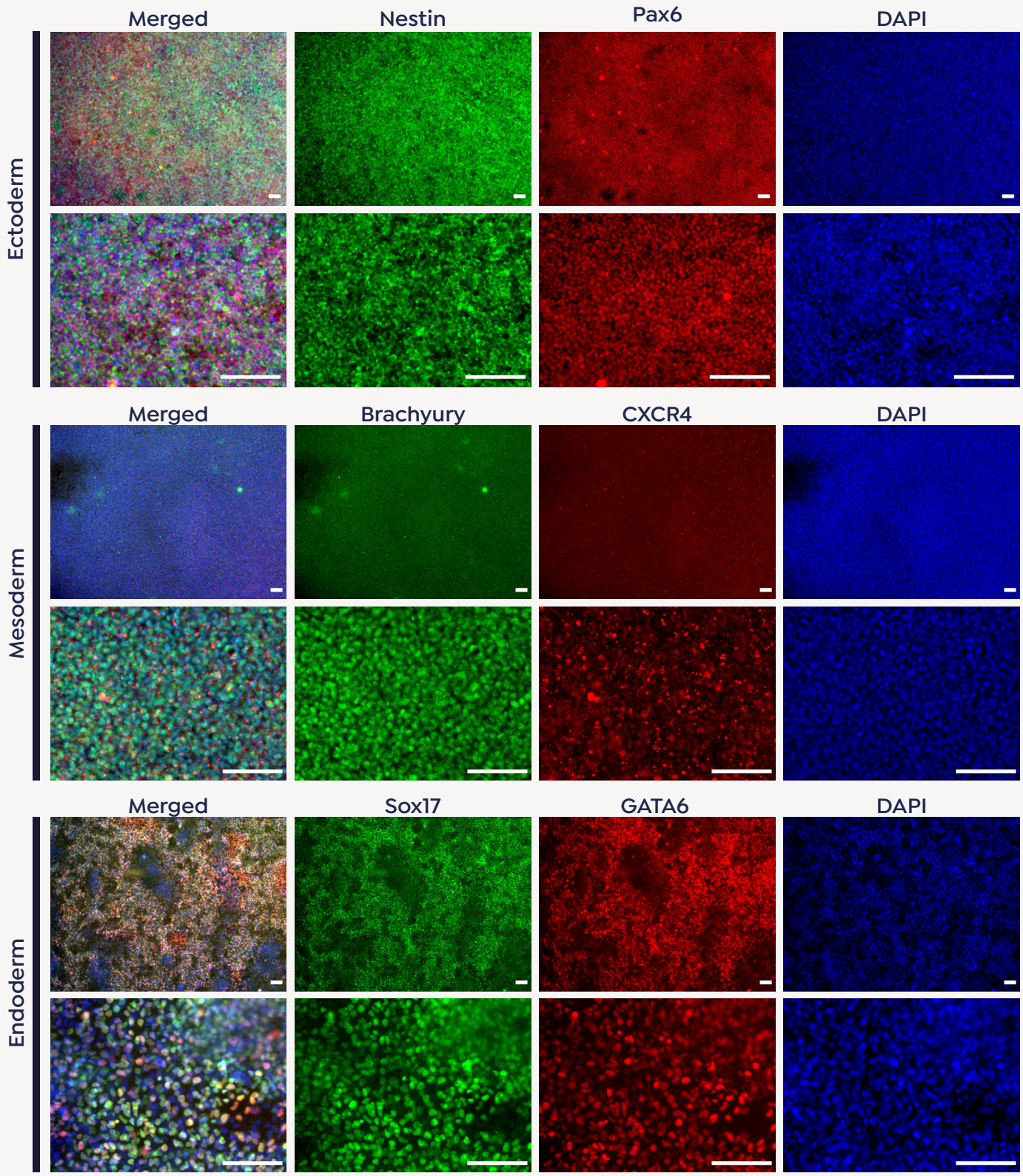

Figure 8. Immunostaining analysis of tri-lineage differentiation.

Figure 8 (continued). Immunostaining analysis of tri-lineage differentiation. Cells grown on myMATRIX iPSC were cultured for over 100 days and then differentiated towards the 3 embryonic germ layers. The differentiation into ectoderm was assessed using Nestin and Pax 6. Mesoderm was detected using Brachyury and CXCR4. Endoderm differentiation was stained for Sox17 and GATA6. Shown are representative images of differentiated cells in an overview image (4x, upper row) and a detail image (20x, lower row). The images reflect an efficient differentiation of the iPSCs into all germ layers after long-term culture.

The myMATRIX family

Stem cells have the remarkable ability to self-renew as well as differentiate into more specialized cell types. This capacity is highly influenced by the cellular microenvironment, which is an organized combination of extracellular matrix (ECM), cells, and interstitial fluid that influence cellular phenotype through physical, mechanical, and biochemical mechanisms. Similar to the ecological niche of an organism, the cellular microenvironment is specific to each cell type. To recreate its complexity on a functional level for ex vivo cell expansion we developed biomatrices that combine ECM components such as glycosaminoglycans (GAGs) with biofunctional peptides. The incorporation of GAGs is beneficial for adhesion-dependent and growth factor-sensitive stem cells and their derivatives. Their ability to bind and stabilize growth factors facilitates the maintenance of stemness and supports differentiation. With our modular technique, we established a library of 96 different microenvironments to screen for biologically relevant compositions for any cell type (screenMATRIX). In addition to the myMATRIX iPSC, we also developed a surface specially formulated for the expansion of human mesenchymal stromal cells (hMSC) in serum-free and xeno-free media - myMATRIX MSC (Thamm et al., 2020).

Description	Size	Cat. No.	Link
screenMATRIX	• 96-well plate	• S1001	https://www.denovomatrix.com/products/screenmatrix
myMATRIX MSC	• T75 flask • T25 flask • 6-well plate	• C0601 • C0701 • C0501	https://www.denovomatrix.com/products/my-matrix-msc
myMATRIX iPSC	• 6-well plate • 96-well plate	• C0505 • C0105	https://www.denovomatrix.com/products/my-matrix-iPSC

Support

denovoMATRIX GmbH is supported and receives funding by the European Union, the European Regional Development Fund (EFRE), the European Social Fund (ESF) and the Free State of Saxony to further develop its denovoMATRIX platform technology for new application areas.

Europäische Union

Europa fördert Sachsen. ESF Europäischer Sozialfonds

Freistaat Sachsen

Europe funds Saxony. EFRE European Regional Development Fund

References

Braam, S. R., Zeinstra, L., Litjens, S., Ward-van Oostwaard, D., van den Brink, S., van Laake, L., Lebrin, F., Kats, P., Hochstenbach, R., Passier, R., Sonnenberg, A., & Mummery, C. L. (2008). Recombinant Vitronectin Is a Functionally Defined Substrate That Supports Human Embryonic Stem Cell Self-Renewal via αVα5 Integrin. Stem Cells, 26(9), 2257–2265. https://doi.org/10.1634/stemcells.2008-0291

Burridge, P. W., Matsa, E., Shukla, P., Lin, Z. C., Churko, J. M., Ebert, A. D., Lan, F., Diecke, S., Huber, B., Mordwinkin, N. M., Plews, J. R., Abilez, O. J., Cui, B., Gold, J. D., Wu, J. C., & Paul Burridge, or W. (2014). Chemically Defined and Small Molecule-Based Generation of Human Cardiomyocytes HHS Public Access. Nat Methods, 11(8), 855–860. https://doi.org/10.1038/nmeth.2999.Chemically

Celiz, A. D., Smith, J. G. W., Langer, R., Anderson, D. G., Winkler, D. A., Barrett, D. A., Davies, M. C., Young, L. E., Denning, C., & Alexander, M. R. (2014). Materials for stem cell factories of the future. Nature Materials, 13(6), 570–579. https://doi.org/10.1038/nmat3972

Chen, G., Gulbranson, D. R., & Zhonggang Hou, Jennifer M. Bolin1, Victor Ruotti1, Mitchell D. Probasco1, Kimberly Smuga-Otto4, Sara E. Howden1, 2, 3, Nicole R. Diol1, 2, 3, Nicholas E. Propson1, 2, 3, Ryan Wagner1, 2, 3, Garrett O, and J. A. T. (2007). Chemically defined conditions for human iPS cell derivation and culture. Nature Methods, 35(1), 144. https://doi.org/10.2307/590562

Gao, L., Nath, S. C., Jiao, X., Zhou, R., Nishikawa, S., Krawetz, R., Li, X., & Rancourt, D. E. (2019). Post-Passage rock inhibition induces cytoskeletal aberrations and apoptosis in Human embryonic stem cells. Stem Cell Research, 41(October), 101641. https://doi.org/10.1016/j.scr.2019.101641

Gilmore, A. P. (2005). Anoikis. Cell Death and Differentiation, 1473–1477. https://doi.org/10.1038/sj.cdd.4401723
Initiative, T. I. S. C. (2012). Screening a large, ethnically diverse population of human embryonic stem cells identifies a chromosome 20 minimal amplicon that confers a growth advantage. 29(12), 1132–1144. https://doi.org/10.1038/nbt.2051.Screening
Kuo, H., Gao, X., Dekeyser, J., Fetterman, K. A., Pinheiro, E. A., Weddle, C. J., Fonoudi, H., Orman, M. V, Jouni, M., Blancard, M., Magdy, T., Epting, C. L., George, A. L., & Burridge, P. W. (2019). Negligible-Cost and Weekend-Free Chemically Defined Human iPSC Culture.
Lambshead, J. W., Meagher, L., Goodwin, J., Labonne, T., Ng, E., Elefanty, A., Stanley, E., O'Brien, C. M., & Laslett, A. L. (2018). Long-Term Maintenance of Human Pluripotent Stem Cells on cRGDfK-Presenting Synthetic Surfaces. Scientific Reports, 8(1), 1–16. https://doi.org/10.1038/s41598-018-19209-0
Ludwig, T. E., Levenstein, M. E., Jones, J. M., Berggren, W. T., Mitchen, E. R., Frane, J. L., Crandall, L. J., Daigh, C. A., Conard, K. R., Piekarczyk, M. S., Llanas, R. A., & Thomson, J. A. (2006). Derivation of human embryonic stem cells in defined conditions. Nature Biotechnology, 24(2), 185–187. https://doi.org/10.1038/nbt1177
Melkoumian, Z., Weber, J. L., Weber, D. M., Fadeev, A. G., Zhou, Y., Dolley-Sonneville, P., Yang, J., Qiu, L., Priest, C. A., Shogbon, C., Martin, A. W., Nelson, J., West, P., Beltzer, J. P., Pal, S., & Brandenberger, R. (2010). Synthetic peptide-acrylate surfaces for long-term self-renewal and cardiomyocyte differentiation of human embryonic stem cells. Nature Biotechnology, 28(6), 606–610. https://doi.org/10.1038/nbt.1629
Peterson, S. E., Westra, J. W., Rehen, S. K., Young, H., Bushman, D. M., Paczkowski, C. M., Yung, Y. C., Lynch, C. L., Tran, H. T., Nickey, K. S., Wang, Y. C., Laurent, L. C., Loring, J. F., Carpenter, M. K., & Chun, J. (2011). Normal human pluripotent stem cell lines exhibit pervasive mosaic aneuploidy. PLoS ONE, 6(8). https://doi.org/10.1371/journal.pone.0023018
Rodin, S., Antonsson, L., Niaudet, C., Simonson, O. E., Salmela, E., Hansson, E. M., Domogatskaya, A., Xiao, Z., Damdimopoulou, P., Sheikhi, M., Inzunza, J., Nilsson, A. S., Baker, D., Kuiper, R., Sun, Y., Blennow, E., Nordenskjöld, M., Grinnemo, K. H., Kere, J., … Tryggvason, K. (2014). Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nature Communications, 5, 1–13. https://doi.org/10.1038/ncomms4195
Rodin, S., Domogatskaya, A., Ström, S., Hansson, E. M., Chien, K. R., Inzunza, J., Hovatta, O., & Tryggvason, K. (2010). Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. Nature Biotechnology, 28(6), 611–615. https://doi.org/10.1038/nbt.1620
Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell, 131(5), 861–872. https://doi.org/10.1016/j.cell.2007.11.019
Takahashi, K., & Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell, 126(4), 663–676. https://doi.org/10.1016/j.cell.2006.07.024
Tapia, N., & Schöler, H. R. (2016). Molecular Obstacles to Clinical Translation of iPSCs. Cell Stem Cell, 19(3), 298–309. https://doi.org/10.1016/j.stem.2016.06.017
Thamm, K., Möbus, K., Towers, R., Segeletz, S., Wetzel, R., Bornhäuser, M., Zhang, Y., & Wobus, M. (2020). A Novel Synthetic, XenoαFree Biomimetic Surface for SerumαFree Expansion of Human Mesenchymal Stromal Cells. Advanced Biosystems, 2000008. https://doi.org/10.1002/adbi.202000008
Vernardis, S. I., Terzoudis, K., Panoskaltsis, N., & Mantalaris, A. (2017). Human embryonic and induced pluripotent stem cells maintain phenotype but alter their metabolism after exposure to ROCK inhibitor. Scientific Reports, 7(October 2016), 1–11. https://doi.org/10.1038/srep42138
Watanabe, K., Ueno, M., Kamiya, D., Nishiyama, A., Matsumura, M., Wataya, T., Takahashi, J. B., Nishikawa, S., Nishikawa, S. I., Muguruma, K., & Sasai, Y. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nature Biotechnology, 25(6), 681–686. https://doi.org/10.1038/nbt1310
Wieduwild, R., Wetzel, R., Husman, D., Bauer, S., El-Sayed, I., Duin, S., Murawala, P., Thomas, A. K., Wobus, M., Bornhäuser, M., & Zhang, Y. (2018). Coacervation-Mediated Combinatorial Synthesis of Biomatrices for Stem Cell Culture and Directed Differentiation. Advanced Materials, 30(22), 1–9. https://