Application Note

AI-assisted Imaging Accelerates Selection of Optimal Cell Culture Matrices

Selecting an optimal extracellular matrix (ECM) product is essential for successful culture of primary cell, stem cells and their derivatives. Manufacturing of these cells further requires defined and safe materials. Here, we present the application of the screenMATRIX, a ready-to-use library of chemical defined ECM mimetics, as a selection tool and show how VAIDR enables visualization and decision making. By combination of both technologies, decisions can be made in a quick, cost-efficient, and objective way while taking into consideration dozens of candidates.

screenMATRIX - a material library tool to identify an optimal matrix

The screenMATRIX is a standardized 96-well plate format that contains a pre-selection of chemically defined compositions of different polysaccharides and synthetic peptides. It can be used to identify optimal microenvironments for a variety of cell types and applications.
screenMATRIX has been applied by cell therapy and cultured meat companies to create custom coatings for animal component-free, large-scale cell expansion, as well as pharmaceutical companies for optimizing in vitro models.

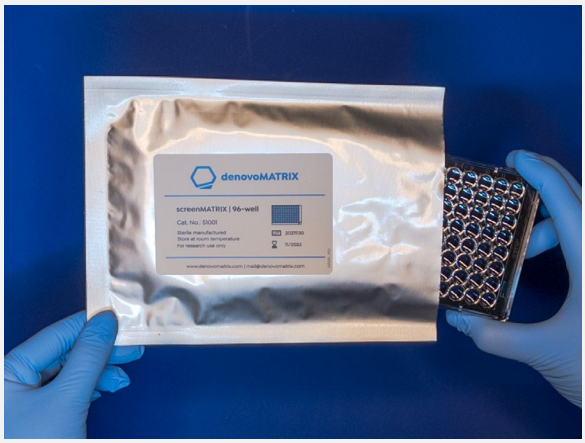

Fig. 1: unpacking screenMATRIX for cell seeding.

VAIDR combines streamlined microscopy with AI-assisted image analysis

VAIDR is an integrated system for automated microscopy and analysis. It combines universally applicable digital phase-contrast microscopy with efficient deep learning algorithms for unbiased pattern recognition and exposes these functionalities in a user-friendly interface, which requires no training in data science.

Fig. 2: VAIDR System composed of plate reader microscope and image analysis station.

Easy imaging and reliable pattern recognition

To illustrate the combinatorial benefit of the screenMATRIX and VAIDR technology in the selection of optimized ECM mimetics, we cultured primary human Mesenchymal Stroma Cells (MSCs) as well as Neuronal Progenitor Cells (NPCs) on screenMATRIX plates for 5 days and 72 hours, respectively, followed by imaging with the VAIDR microscope. Images were taken in phase-contrast mode without additional cell staining (Fig. 3A). Then, the VAIDR imaging algorithm was trained to recognize nuclei and cytoplasm of the MSCs as well as cell centers, cytoplasm, and neurites of the NPCs by manually labeling each component several times. The result, automated recognition of the cell bodies (green) and nuclei (magenta) of both cell types in all images, are shown in colorized images of Fig. 3B-D. The VAIDR algorithm reliably identified the individual components across all acquired images. Variations in cell morphology (from stretched to compact) can be also recognized by the VAIDR algorithm. Thus, VAIDR enables quick and unbiased analysis of morphological cell features without the need for manual sample preparation such as cell fixation and staining.

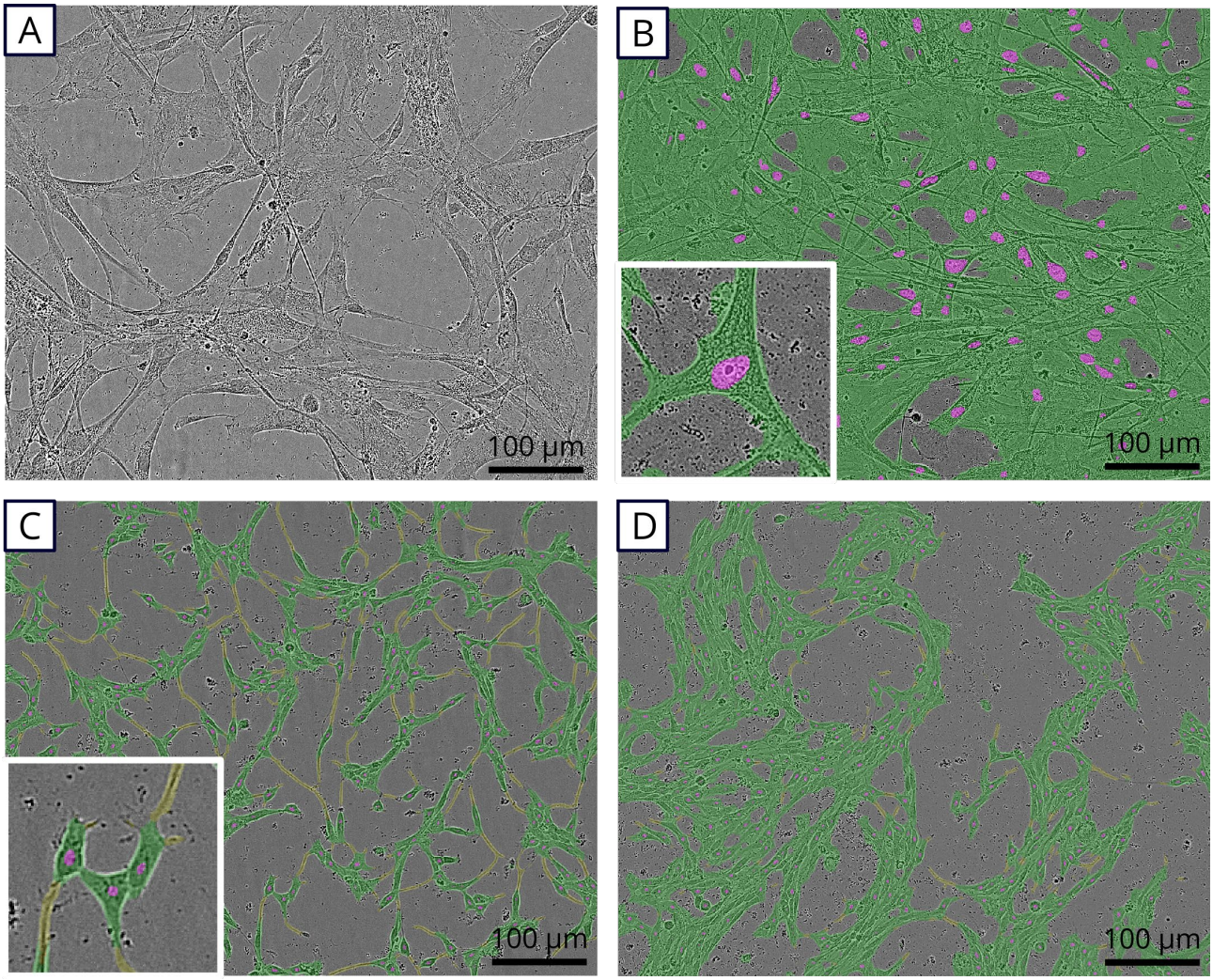

Fig. 3: Images of MSCs and NPCs cultured on the screenMATRIX automatically taken and analyzed with VAIDR system. A) Phase contrast image of MSCs, B) MSCs with automatically detected cytoplasm (green) and nuclei (magenta), C) NPCs of well E1 with spread morphology and D) NPCs of well H12 with compact morphology both with highlighted centers (magenta), cell bodies (green) and neurites (yellow).

Unbiased data analysis using AI

Cell adhesion and growth is one of the primary selection criteria to identify a suitable cell culture matrix, which can be quantified by counting the number of cells or by calculating the area fraction covered by cells (confluency). We performed both analyses using the results from the automated detection of the VAIDR algorithm. To quantify the number of cells, we counted the number of nuclei denoted as "nucleus" for MSCs or of the cellular "center" for NPCs. For the evaluation of confluency, we computed the area fraction that was covered by any cell-related structure, i.e. cytoplasm, nuclei, and neurites. The cell count results for NPCs are illustrated in Fig. 4. The heat map displays a distinct pattern that reflects preferred and unfavored microenvironments for NPCs on the screenMATRIX with respect to cell attachment and growth.

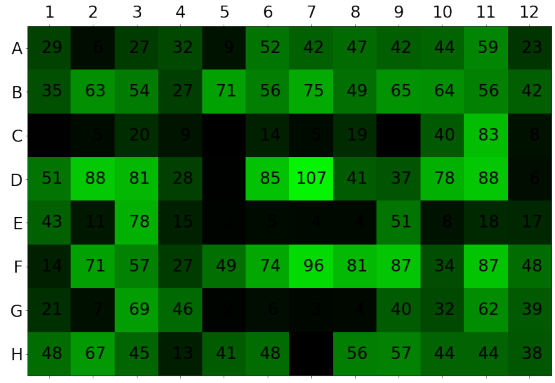

Fig. 4: Evaluation of confluency of NPCs grown on the screenMATRIX is displayed as cell density (in 1000 cells/cm²). For details about the screenMATRIX,setup view the product information sheet[1].

To validate these results, we compared them to the standard operating procedure, which includes the fixation and staining of the cells to label nuclei and cell bodies with fluorescent dyes. The stained cells were imaged with a fluorescent microscope and the resulting images are analyzed for number of nuclei and confluency comparable to the approach in our study. When comparing cell-growth-hits, defined as cell count or confluency in the upper quartile of all wells, we found an overlap of over 83% between both methods.

Simple detection of preferred morphologies

Different cell types express specific phenotypes. Hence, another important criterion for the selection of an optimal ECM-mimetic product is the identification of cell morphologies fulfilling the typical patterns and thereby meeting the quality criteria. For example, NPCs need to maintain their precursor stage and should not mature into neurons. One key indicator for this unwanted maturation is cellular stretching as an early indicator for formation of neurites.

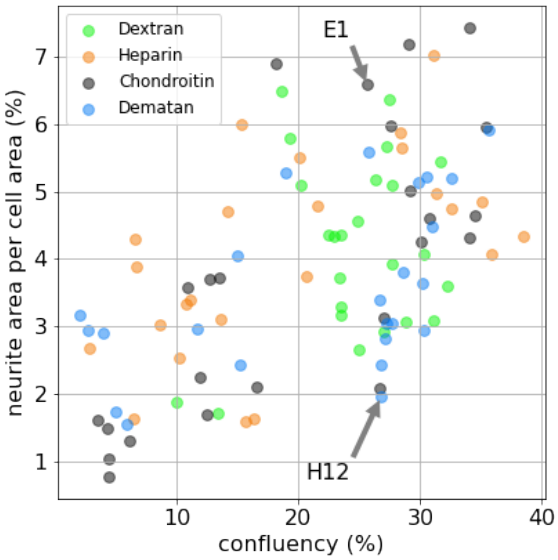

Fig. 5: NPC neurite fraction vs confluency dot plot.

[1] https://www.denovomatrix.com/wp-content/uploads/2018/11/screenMATRIX-info-sheet-S1001-V2.pdf

We therefore utilized the capability of the VAIDR system to detect and quantify subcellular structures. We detected the neurites and computed the area fraction covered by neurites with respect to the total cytoplasmic area.
The results shown in the dot plot of Fig. 5 provide several insights. For example, the neurite density increases with confluency, but for any given confluency, the neurite density varies in a wide range depending on the ECM-mimetic they were grown on. To illustrate the large variations in neurite density, we highlighted well E1 and H12 (Fig. 5). In both wells, the cells grew to intermediate confluency, but NPCs grown in E1 show significantly more neurites compared to those in H12 (Fig. 3). As illustrated by the mixture of colored markers in Fig. 5, the polysaccharide components of the screenMATRIX are distributed across the entire data range, except for dextran sulfate, which is concentrated in the high confluency region of the plot. As a result of the data visualization and evaluation with the VAIDR technology potential high performing candidates showing a high degree of confluence and low neurite per area count similar to the H12 composition could be identified. Thus, by combining the read-out of cell growth and formation of neurites, a **more data-based decision** on suitable matrix compositions can be made.

Conclusion

We have illustrated a workflow for a data-based ECM product selection from 96 different candidates on the screenMATRIX using the VAIDR system for imaging and analysis. The example application presented here highlights the advantages of VAIDR's live-cell compatible, label-free imaging and analysis technology in terms of power and cost efficiency.

In summary, the presented combination of both technologies **accelerates and simplifies** the identification of optimal ECM-mimetics and growth conditions for primary and stem cells by enabling **objective decision making**.

Bruno Chilian, Director at
TRI Thinking Research Instruments GmbH
bruno.chilian@vaidr.de
www.vaidr.de

Richard Wetzel, Managing Partner at
denovoMATRIX GmbH
wetzel@denovomatrix.com
www.denovomatrix.com