Melt Electrowritten In Vitro Radial Device to Study Cell Growth and Migration

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The development of in vitro assays for 3D microenvironments is essential for understanding cell migration processes. A 3D-printed in vitro competitive radial device is developed to identify preferred Matrigel concentration for glioblastoma migration. Melt electrowriting (MEW) is used to fabricate the structural device with defined and intricate radial structures that are filled with Matrigel. Controlling the printing path is necessary to account for the distance lag in the molten jet, the applied electric field, and the continuous direct-writing nature of MEW. Circular printing below a diameter threshold results in substantial inward tilting of the MEW fiber wall. An eight-chamber radial device with a diameter of 9.4 mm is printed. Four different concentrations of Matrigel are dispensed into the radial chambers. Glioblastoma cells are seeded into the center and grow into all chambers within 8 days. The cell spreading area demonstrates that 6 and 8 mg mL\(^{-1}\) of Matrigel are preferred over 2 and 4 mg mL\(^{-1}\). Furthermore, topographical cues via the MEW fiber wall are observed to promote migration even further away from the cell seeding depot. Previous studies implement MEW to fabricate cell invasive scaffolds whereas here it is applied to 3D-print in vitro tools to study cell migration.

Tissue regeneration, inflammation, and cancer progression are being considered as the frontline in several tumor and tissue engineering paradigms, and cell migration is an essential part of these scenarios.\(^{[1]}\) Migration is influenced by chemotactic, topographical, and mechanotransductive cues from the extracellular matrix.\(^{[2]}\) Moreover, cell migration in the context of cancer metastasis is a complex and important factor for understanding tumor progression.\(^{[3]}\)

The most aggressive form of a primary brain tumor is glioblastoma multiforme (GBM) which are highly invasive heterogenous tumors with a very low survival rate.\(^{[4]}\) Surgical resection and chemotherapeutic or radiotherapy is commonly used for patient treatment, however, tumor recurrence is very frequent. Importantly, GBM cells invade and migrate along white matter tracts and brain blood vessels which promote tumor dissemination.\(^{[5]}\) Hence, it is critical to understand the basic process of tumor migration and progression in order to develop new therapeutic drugs and treatment regimens.

While therapeutic approaches that minimize GBM migration are logical, another approach focuses on guiding these cells away from the tumor into biomaterial reservoirs with the goal to reduce tumor size.\(^{[6]}\) This diversional approach is based on the placement of a tube filled with a matrix and oriented substrate that provides topographical guidance cues at the tumor site. This results in the attraction and guidance of migration of GBM cells into the tube, effectively reducing overall tumor size. Therefore, in line with this study and the fact that GBM cells have an affinity for white brain matter and blood vessels, it is important to develop new 3D in vitro cell culture models to determine the optimal matrix composition that drives GBM migration.

Novel research methods and tools provide an opportunity to study cell migration during cancer metastasis where loss of cell adhesion from the primary tumor along with increased cell motility and invasion occurs. There is evidence from 3D microfluidic devices and microchips that matrix stiffness influences the migratory and invasive capabilities of tumor cells through the structure characteristics.\(^{[7]}\)

There have been significant advances to understand the process of cell migration using in vitro models, which are cost effective and easier to use compared to in vivo studies. With existing in vitro assays, cell migration conditions are well-defined with many based on the traditional 2D cell culture methods.\(^{[8]}\) While simple to use, they are challenged to recapitulate the 3D in vivo microenvironment.
Many assays have been developed to provide additional information, such as the response of cells to biochemical, adhesive, topographical,[10] and mechanical[11] cues. The scratch assay, used widely for migration analysis, consists of analyzing the movement of a monolayer of cells after a pipet tip is dragged along the surfaces.[12] Although this method is simple to use, scratch assays are not always comparable to the physiological conditions. In contrast, 3D migration environments would provide a better realization and analysis of cell migration by incorporating chemotactic stimuli or topographical cues.[13] The benefits of developing a competitive in vitro migration assay are to test a spectrum of different microenvironments at the same time.

Recent progress in additive manufacturing (AM) provides new fabrication opportunities for in vitro migration models in terms of reproducibility, precision, minimal volume of materials, well characterized microenvironment, and quantifiable gradient generation.[14] AM technologies such as fused deposition modeling, stereolithography, and melt electrowriting (MEW) facilitate faster prototyping, short times for manufacturing, and lower manufacturing cost.[15] MEW is an AM technology that uses sub-micron to micron scale diameter fibers as a building block while controlling their orientation,[16] generating structures with precise placement of fibers and high surface volume ratio.[17] Depending on the laydown pattern and inter-fiber spacing, MEW scaffolds with different porosities and pore designs such as regular squares, dodecagon, triangular/hexagon, and octagon can be fabricated.[18] The fabrication of MEW structures with an increased build height might be applicable for in vitro systems. The current maximum thickness of fabricated MEW scaffolds is 7 mm.[19]

For MEW, a molten polymer is delivered to a nozzle and stretched into low-micron diameter fibers by applying an electrical field. The fiber size depends on several parameters such as nozzle diameter, pressure, and collector distance, which is the distance between the nozzle and the collector.[14] The collector speed can affect the lag in the electrified molten jet and is therefore an important variable to control the accuracy of MEW fiber placement. The threshold collector speed that incorporates these parameters was named critical translational speed (CTS).[20] The vast majority of MEW scaffolds have been fabricated above the CTS mostly as straight fibers and box-like structures, as it is difficult to predict where the fiber will land when the collector is changing directions. Herein, we systematically investigated the influence of collector speed and number of layers on the accuracy of circular-shaped constructs to extend the design perspective for MEW. The goal of this investigation was to create a MEW in vitro competitive 3D radial device toward the rapid screening of different hydrogels and/or hydrogel concentrations/stiffness to test cell migration of the human glioblastoma cell line U87. This 3D radial device could also be adapted with different hydrogels and other cell types for future testing.

The structural frame of the competitive 3D in vitro radial culture device was made using MEW of poly(e-caprolactone) (PCL). The small dimensions that we aimed to use were not currently possible with conventional extrusion-based technologies, or stereolithography, which have lower resolutions than MEW. However, since most MEW research is based on linear fiber deposition, fiber placement in this study is affected by continual changes in the direct-writing direction. Due to the need to control the molten jet lag, fiber placement under these circumstances was especially exacerbated by fiber pulsing phenomenon (Figure 1A). Therefore, very stable printing parameters (Table S1, Supporting Information) were necessary to achieve MEW fiber placement accuracy. The MEW printing conditions used here resulted in a fiber diameter of 17.55 ± 0.87 µm and the collector speed used was 1.4xCTS to print the structural frame of the competitive 3D in vitro radial culture device.

Glass slides were coated with star-shaped NCO-poly(ethylene oxide-stat-propylene oxide) (sP(EO-stat-PO)) except for a 3 mm circle at the center of the device. This coating ensured the adhesion of the fibers during consequent washing and handling. Each radial device was composed of a central cell depot (which was devoid of the hydrophilic sP(EO-stat-PO) coating) and radially expanding matrix chambers (Figure 1).

One general limitation of MEW addressed in the current design was that fiber direct-writing is a continuous process and starting/stopping of the jet mid-print was not practically implementable. A continuous path (Figure 1A) was developed so that each printed layer had a similar height to the next. Several different design reiterations using a continuous printing path (Figure S1, Supporting Information) were investigated. The placement of cells in the central depot sealed the containers and allowed different matrices to be dispensed and form a hydrogel within each chamber of radial culture device (Figure 1B).

A 3D structure is produced by stacking the printed single fiber in a layer-by-layer approach. Since the collector speed is 1.4xCTS, the electrified molten jet lands on the collector behind the nozzle position. The designed radial culture devices contain multiple direction changes, so it is critical to compensate the jet lag to attain final reproducible structures. Therefore, we

**Figure 1.** Overview of the competitive in vitro radial culture device. A) Schematic of the MEW jet with the blue circle demonstrates a Taylor cone of the molten polymer and the implications of fiber pulsing on jet lag (adapted under the term of Creative Commons 3.0 License.[20] Copyright 2016, The Authors, published by De Gruyter and adapted under the term of Creative Commons 3.0 License.[21] Copyright 2018, The Authors, published by IntechOpen.). B) Overview of the radial device design with the cell depot in the center (light blue) surrounded by eight chambers used for matrix testing. In the present study, different concentrations of Matrigel (red) were used.
investigated the influence of collector speed and the number of printed layers (i.e., wall height) on accuracy, precision, and dimensional consistency of the radial migration device. The determination of the dimensional differences in printing path, actual fiber placement, and the build height was therefore required.

The printing accuracy ($D_{ACC}$) indicates how the actual maximal circular diameter ($D_A$) approximates the printing path of the nozzle, or the designed diameter ($D_D$). To determine the printing accuracy, a series of circular structures with a diameter size from 2 to 16 mm was printed and assessed at different speeds (Figure 2). There was a clear improvement in the printing accuracy with larger circular paths (Figure S2A,B, Supporting Information) as well as with reduced collector speed due to the growing lag between the nozzle and the jet contact point. In contrast, circles with a 16 mm diameter did not show any significant difference at these different speeds ($p_{1CTS-2CTS} = 0.967, p_{1CTS-3CTS} = 0.096, p_{2CTS-3CTS} < 0.001$). $D_{ACC}$ values for 16 mm were $94.6 \pm 2\%$, $94.1 \pm 1\%$, and $93.1 \pm 1\%$ at 1CTS, 2CTS, and 3CTS, respectively.

The effect of increasing the number of layers on the $D_{ACC}$ at 2CTS was also investigated for 10, 20, and 30 layers (Figure 2C). The 2 mm diameter of circular structures exhibited no significant difference on the $D_{ACC}$ with increased layers. The accuracy, however, of 4, 8, 12, and 16 mm diameter circles was significantly different at 20 layers compared to 10 and 30 layers (Figure 2C). Interestingly, the small diameter circles were cone-shaped, especially at higher collector speeds (Figure 2A). At collector speeds above the CTS, the inward tilting of the fiber wall was previously observed for sinusoidal MEW structures. However, the previous study did not investigate the effect of the printing speed and the number of layers on inward tilting of walls. Such inward tilting of the wall into a cone shape can be
measured by comparing the diameter of the first printed layer to the diameter of the top printed layer. The variability of different diameters was calculated using the following formula (1) in order to compare the inward tilting of the circular structures

\[ T_i = \frac{D_b - D_t}{D_b} \times 100 \]  

(1)

where \( T_i \) is the inward tilting index, \( D_b \) is the bottom, and \( D_t \) is the top layer diameter. Figure 2D shows the influence of the collector speed on \( T_i \) for different circle diameters using 20 layers printed at 1xCTS, 2xCTS, and 3xCTS, where \( T_i \) decreased with increasing circle diameter. \( T_i \) values were 22 ± 2%, 6.9 ± 1%, 1.4 ± 0.9%, 1.6 ± 1%, and 1.07 ± 0.7% at 1xCTS for 2, 4, 8, 12, and 16 mm circles. No significant differences were observed between \( T_i \) values at 1xCTS, 2xCTS, and 3xCTS of circles with a diameter of 12 and 16 mm. Next, the consequences of decreased and increased layers of the circular structures and the printing speed on dimensional accuracy were evaluated (Figure 2E). \( T_i \) values revealed no significant differences between 10, 20, and 30 layers for 12 and 16 mm diameter circles.

The height of a MEW construct is expected to correspond to the number of layers multiplied by the fiber diameter. However, we found that fiber layers fuse differently depending on the collector speed. Concomitantly, the jet lag increased and affected jet cooling. As a consequence, fiber layer fusion led to decreased construct height compared to the expected height. The relative error of the expected height versus the actual height for 10 mm diameter circular structures at 2xCTS and 1.4xCTS for 10, 20, and 30 layers is presented in Figure 2F. An increase in the number of fiber layers resulted in a larger difference between the expected and actual heights at least for a collector speed of 1.4xCTS. For the ten-layer group, the relative error of height of circular structures at 2xCTS is similar to 1.4xCTS. However, the relative error of the circles for 20 and 30 layers at 2xCTS is significantly lower than 1.4xCTS. The relative error of 10 mm scaffolds at 2xCTS is 10.2 ± 2% and 9.9 ± 4% for 20 and 30 layers, respectively. Since the collector distance does not change during printing, it is possible that with increased build height melting of the upper layers might occur due to increased proximity to the MEW head. Recently, Wunner et al. elaborated that the distance between the top layer and the printing head can be kept constant by adjusting the z-axis while increasing the voltage to produce superior layering. Adopting such parameters to our approach might preserve the electrostatic force, improve fiber stacking, and prevent excessive fusion between layers.

For the cell assay, three different groups (10 layers, 20 layers, 30 layers) of migration devices were characterized and compared (Figure 3). Representative scanning electron microscope (SEM) images of the three different scaffolds with their turning points created by the MEW jet for different layers are shown in Figure 3A–I. Based on the printing outcome and in line with the goal to minimize the size of the migration device, the final cell culture system had a diameter of 9.4 ± 0.1 mm, a wall height of 30 layers (around 350 µm), and a wall thickness of 17 ± 1 µm. Additionally, the internal reference rings were introduced every ten layers at a spacing of 2 mm apart to provide a visual guide and prevent the dispensed cells from being drawn into the chambers due to surface tension (Figure 4A and Figure S2D, Supporting Information).

A human glioblastoma cell line, U87, was used to assess the competitive 3D in vitro radial culture device with respect to cell growth and migration. Different Matrigel concentrations within separate chambers facilitated U87 cells to determine a preferred matrix for migration and growth. A total of 30 000 cells in 4 mg mL\(^{-1}\) of Matrigel were seeded as a droplet into the center of the pre-warmed MEW device (Figure 4A) to seal off the chambers. Four increasing concentrations (2, 4, 6, and 8 mg mL\(^{-1}\)) of cell-free Matrigel were added into the eight surrounding chambers of the radial devices (Figure 4D), and U87 cells were cultured for 8 days. At day 8, the cytoskeletal protein β-actin and the nuclei of the U87 cells were stained and then reconstructed.
into 3D images (Figure 4B and Video 1, Supporting Information). U87 cells formed a dense cellular network as shown in an example from a chamber containing 6 mg mL\(^{-1}\) Matrigel (Figure 4C). Each matrix has a neighboring chamber with an identical matrix concentration, where the MEW fiber wall could be assessed for topographical migration. The areas of cell distribution were monitored at days 1, 4, and 8 (Figure 4D–D3). The lowest percentage of cells was present in 2 mg mL\(^{-1}\) of Matrigel (14 ± 2.8%, Figure 4E). The calculated areas of U87 cells at different days after seeding from six independent experiments (n = 6) showed a strong preference toward 6 and 8 mg mL\(^{-1}\) of Matrigel (33 ± 6.3% for 6 mg mL\(^{-1}\), 31.2 ± 8.4% for 8 mg mL\(^{-1}\), Figure 4E). The mean values demonstrate a significant increase of tumor cell growth at 6 mg mL\(^{-1}\) compared to 2 mg mL\(^{-1}\) (\(p < 0.05\); Figure 4E). It is notable that there is also some topographical guidance of the cells due to the MEW fiber walls, Figure 4D1–D3).

The importance of matrix stiffness for brain tumor growth has been shown in various reports.\(^{[23]}\) For glioblastoma, it has been demonstrated that matrix stiffness influences morphology, proliferation, and migration.\(^{[24]}\) Similar effects on morphology and proliferation have also been described for primary
cells of the brain, e.g., microglia and astrocytes. The matrix used in the present study, up to 8 mg mL$^{-1}$ of Matrigel, is very soft as determined previously [26] ($66 \pm 4.4$ Pa for 8 mg mL$^{-1}$ and $31 \pm 5.6$ Pa for 4.5 mg mL$^{-1}$). The elastic modulus of 6 mg mL$^{-1}$ Matrigel was assigned with $48 \pm 9.2$ Pa; $n = 3$ and is similar to values reported for the normal brain, whereas tumors have been found to prefer material with a stiffness above 100 Pa.[27] Enhanced growth and migration of the glioblastoma cell line U87 noted at higher Matrigel concentrations (6 and 8 mg mL$^{-1}$) is reflected in our radial culture device, although biochemical signals also play a role. The calculation of the invasion front at different Matrigel concentrations reflects the data from cell growth. The furthest distance (in mm) where cells have migrated/proliferated from the center in 8 days was observed at 6 mg mL$^{-1}$ Matrigel ($3.2 \pm 0.4$ mm compared to $1.9 \pm 0.2$ mm with 2 mg mL$^{-1}$, $p < 0.05$; $2.4 \pm 0.5$ mm for 4 mg mL$^{-1}$, and $2.6 \pm 0.4$ mm for 8 mg mL$^{-1}$, both did not reach significance compared to 2 mg mL$^{-1}$). The use of our radial device together with differential extracellular matrix densities and compositions in future experiments—especially defined matrices with immobilized cues—will allow the identification of formulations which either further increase or decrease the tumor migration behavior. The encouragement of GBM cells to migrate was part of certain biomaterial-based paradigms to reduce brain tumor volume in vivo.[9] To enhance spatio-temporal resolution of the cellular processes, a combined approach using automation of the matrix dispensation in the radial device together with real-time measurements to study the migratory capability of the cells and a quantitative approach to investigate changes in gene expression profiles are needed to better understand tumor cell dynamics. Besides dissecting cell–matrix interaction, the radial device is further suitable to investigate the migratory behavior of cells upon co-culturing with other cell types present in the in vivo situation.[28,31]

MEW remains a young AM technology, that is currently focusing on the fabrication of scaffolds or matrix-reinforcing structures for tissue engineering or biofabrication. However, MEW could also be used for the fabrication of precise and advanced in vitro systems. The limits of MEW resolution in this context reported here give insight where this AM technology can potentially contribute to establishing improved 3D in vitro assays, including radial migration. With further automation and experiment digitization, this newly developed MEW 3D in vitro radial cell culture device may be suitable to investigate cellular migration, and could facilitate rapid screening and reiteration of different hydrogels in a competitive manner. The presented methodology has applicability to other cell types to study individual cell migration parameters such as cell trajectories, accumulated distance, and speed. Initially, our in vitro model provides a tool for analyses of glioblastoma migration in 3D environments composed of different hydrogels, encouraging future studies with co-cultures or other applications such as drug screening. Due to the ability of soluble cues to migrate between chambers, however, future designs would include matrices or fibers with tethered bioactive molecules,[40] so that clear distinctions between chambers are easier to elucidate.

In this study, MEW was used to fabricate a 3D radial migration device in order to establish a competitive assay for glioblastoma cell migration. Fabricating such a radial migration device using MEW, however, required a constant change in the printing path for MEW, while maintaining a continuous fiber during the printing process. With the current design of the radial device, we determined the printing effects on the radius of the final dimensions. Moreover, we showed the influence of the collector speed and wall height to the fabrication accuracy. The radial device was successfully established and functionally tested using a glioblastoma cell line implementing different matrix concentrations. Such a competitive migration device is applicable for other cell types and we predict that, when further automated, it could represent a useful tool to screen a spectrum of different matrices.

**Experimental Section**

**Materials:** Pellets of medical-grade PCL (Corbion Inc., Netherlands, PURASORB PC 12, Lot#1712002224 05/2018) were used after appropriate storage.[31] The chemically reactive macromer, six-armed star-shaped NCO-terminated sP(EO-stat-PO) was provided by DWI Leibnitz Institute for Interactive Materials (Aachen, Germany) with a molecular weight of 12,000 g mol$^{-1}$.

NCO (sP(EO-stat-PO))-Coated Glass Slides: MEW constructs for the radial device were printed onto a glass slide coated with a chemically reactive surface based on sP(EO-stat-PO) as previously described.[34] except for the center (3 mm in diameter) of the radial device. Briefly, glass slides were cleaned by acetone, distilled water, and isopropanol for 5 min followed by air drying. Then, the slide was activated with oxygen plasma treatment in the plasma generator (Pico low pressure plasma system; Diener electronic, Ebhausen, Germany) in a vacuum of 0.3 mbar for 20 min. Afterwards, slides were placed in a desiccator with 3-aminopropytrimethoxysilane for surface activation. Slides were then removed and 10 mg mL$^{-1}$ sP(EO-stat-PO) in 10% tetrahydrofuran solution in MilliQ water was loaded onto the glass slides and distributed with rotational spin coating homogeneously at 2500 rpm for 40 s.

**Design Optimization and Printing of the MEW Radial Device:** The structural frame of the radial migration device was printed using an in-house built MEW machine[37] which was based on a x-y-z stage system and controlled via a G-code (A3200 Motion, version 4.09.000.0126, Aerotech Inc., Pittsburgh, USA). An electrical heater was utilized that contained a 22-gauge nozzle with Luer Lock (Carl Roth, Germany) and a syringe filled with PCL. The final parameters were a melt-temperature of 90 °C, an applied voltage of 5.5 kV, a pressure of 0.4 bar, and a collector distance of 3 mm. A pressure control valve (SMC, Germany) was operated with air for delivering the molten polymer to the nozzle. The structural MEW-frames were printed onto sP(EO-stat-PO)-coated glass slides except for the center of the cell depots using parameters previously described for circular constructs, combined with a collector speed of 1.4×CCTS. Internal reference rings were introduced into the G-code at a spacing of 2 mm apart smaller circles every ten layers. The CTS was determined at 90 °C for a single-layer circular pattern. Circles were MEW between 1×CTS and 3×CTS with 2, 4, 8, 10, 12, and 16 mm diameters at 10, 20, and 30 layers to determine the printing accuracy. The actual diameters and heights were exhibited using the stereomicroscope software (Zen 2.3, Discovery V20, Carl Zeiss Microscopy GmbH, Göttingen, Germany). For each circle, five lines were drawn through the center. The average determined from five lines was used as the actual diameter of the circle. The effect of layer number on the height of the printed structures was investigated. Images of the different structures harboring different numbers of layers (10, 20, 30 layers) were taken by using stereomicroscope. The height of the printed structures was determined using the stereomicroscope software (Zen 2.3). For every circle, perpendicular lines were drawn of which the average was used as the mean height of the structures.

**Imaging of Radial Cell Culture Devices:** A stereomicroscope (Discovery V20, Carl Zeiss Microscopy GmbH, Göttingen, Germany) and a Crossbeam 340 scattering electron microscope equipped with GEMINI...
Migration Device Assembly and Cell Seeding: The U87 cell line was obtained from ATCC (HTB-14; ATCC – Global Biosource Center, Manassas, VA, USA). U87 cells were grown in Minimum Essential Media (MEM, Life Technologies, Darmstadt, Germany). The medium was supplemented with glutaMAX (200 × 10−6 m) and sodium pyruvate (100 × 10−6 m), penicillin (50 U mL−1)/streptomycin (50 µg mL−1), and 10% fetal calf serum. Cells were grown in an incubator at 37 °C and 5% CO2. Cells were split twice a week using trypsinization with accutase.

Prior to seeding, radial devices were subjected to UV light for 20 min and washed once with 70% ethanol and afterward with D2O. A total of 30 000 cells were mixed with Matrigel (Corning, NY, USA) (final concentration 4 mg mL−1) and placed as a drop into the center of the radial device in a total volume of 6 µL. The cell-laden droplet was allowed to polymerize for 2 min at 37 °C. Following polymerization of the cell-laden droplet in the center of the radial device, Matrigel (6 µL for each chamber) at four different concentrations (2, 4, 6, 8 mg mL−1) was dispensed in eight different chambers surrounding the cell plaque and incubated at room temperature in the cell culture hood for 2 min to allow polymerization at 37 °C. Following polymerization, the device was filled with 7 mL of MEM medium. The growth of the cells was documented within each chamber at days 0, 1, 4, and 8 after seeding.

Immunocytochemical Staining: Cells were fixed using 2% paraformaldehyde for 20 min at 21 °C. Following three washing steps with phosphate-buffered saline (PBS), cells were permeabilized by PBSt, 5% bovine serum albumin (BSA), 0.1% Triton-X-100. Cells were incubated with ActinGreen 488 ReadyProbes reagent (1:50 in PBS, 5% BSA, R37110, Thermo Fischer Scientific, Darmstadt, Germany) for 2 h at 21 °C. Cells in the radial device were washed three times with PBS. Stained cells were mounted using ProLong Glass Antifade Mountant with NucBlue (Thermo Fischer Scientific, Darmstadt, Germany). Images were taken with an Olympus microscope (Fluoview ix1000, Olympus, Hamburg, Germany). Merged image stacks of 1 µm (62 total) were used to prepare a video of U87 cells in the 3D surrounding. Imaris 7.7.2 was used for 3D reconstruction (Oxford Instruments, Abingdon, UK).

Experimental Design and Statistical Analysis: MEW radial devices: Dmax and T values of the circles were compared using the Tukey’s multiple comparisons test (one-way analysis of variance (ANOVA)) (Origin Pro, 2018b, OriginLab Corporation, Northampton, MA, US). Unpaired t-test was performed to compare the relative error of the height between different speeds. The three different independent samples were printed and a total of 15 diameter measurements were performed for each speed. Statistical significance was determined using a one-way ANOVA test (p-values below 0.05 were considered significant).

Experiments including cells were performed in six independent biological replicates (unless otherwise stated). The number of experiments for analyses were presented in the legends to the appropriate figures.

Microscopy, image acquisition: Images from grown cells were taken using a Leica DM IL LED (Leica, Wetzlar, Germany) microscope together with a Samsung mobile device 16 Megapixel (CMOS), F1.9-Blend. For analyzing cell spreading, the radial device was divided into nine areas: the center and eight radial sections containing different concentrations of Matrigel. From each section, a picture was taken. The combined images gave rise to an entire radial device at the timepoints investigated (days 1, 4, and 8). The images were further developed and organized by Adobe Photoshop and Illustrator software (Adobe) or Corel Draw (Corel Draw X6).

Image analysis for quantification: processing of images using ImageJ[18].

For each quarter (two chambers contain the same Matrigel concentration) of one radial device, the total size was measured by framing the quarter manually with the area selection tool. The pixel number of one quarter was set to 100%. Within each quarter, the area containing cells were measured by framing (area selection tool). The corresponding number of pixels was taken to calculate the percentage compared to the total area (relative cell growth [%]).

The cell invasion front was determined by the distance from the center to the farthest point that cells were migrated/proliferated at day 8 after seeding. The parallel dimension tool (Corel Draw X6) was used to calculate the distance (in mm). For each quarter (with two chambers always containing the same Matrigel concentration) of one radial device, a total of five measurements were performed (see Figure 4F).

The mean value for the invasion front was further calculated using GraphPad Prism Software. Quantification of data obtained from cell distribution analysis and invasion front determination within the radial devices were compared using the unpaired two-tailed t-test (GraphPad Prism, GraphPad Software, San Diego, CA, USA) with a probability of error of p < 0.05 was considered significant.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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