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Multifunctional polymer scaffolds with adjustable pore size and chemoattractant gradients for studying cell matrix invasion

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ABSTRACT

Transmigrating cells often need to deform cell body and nucleus to pass through micrometer-sized pores in extracellular matrix scaffolds. Furthermore, chemoattractive signals typically guide transmigration, but the precise interplay between mechanical constraints and signaling mechanisms during 3D matrix invasion is incompletely understood and may differ between cell types. Here, we used *Direct Laser Writing* to fabricate 3D cell culture scaffolds with adjustable pore sizes (2–10 μm) on a microporous carrier membrane for applying diffusible chemical gradients. Mouse embryonic fibroblasts invade 10 μm pore scaffolds even in absence of chemoattractant, but invasion is significantly enhanced by knockout of lamin A/C, a known regulator of cell nucleus stiffness. Nuclear stiffness thus constitutes a major obstacle to matrix invasion for fibroblasts, but chemotaxis signals are not essential. In contrast, epithelial A549 cells do not enter 10 μm pores even when lamin A/C levels are reduced, but readily enter scaffolds with pores down to 7 μm in presence of chemoattractant (serum). Nuclear stiffness is therefore not a prime regulator of matrix invasion in epithelial cells, which instead require chemoattractive signals. Microstructured scaffolds with adjustable pore size and diffusible chemical gradients are thus a valuable tool to dissect cell-type specific mechanical and signaling aspects during matrix invasion.

1. Introduction

Cells embedded in tissues are exposed to a complex three-dimensional (3D) and multifunctional microenvironment containing multiple extracellular matrix (ECM) components, other cell populations and soluble or ECM-bound cellular signaling factors. Cells constantly modify and restructure this environment and often display directional migration along a concentration gradient of bioactive signaling molecules [1,2]. For instance, directed migration is crucial for wound healing and the immune response as it enables immune cells to reach their targets [3–5]. Furthermore, cell migration through tissues is involved in many pathological processes, such as chronic inflammation and cancer cell metastasis [6,7]. In these cases, different aspects of cell behavior are also strongly influenced by the ease with which cells are able to migrate through the tissue microenvironment. For instance, the malignancy of cancer is, in parts, determined by the degree to which tumor cells can invade neighboring tissues or distant organs [8–10]. In many cell types, interphase nuclei are about two to ten times stiffer than the surrounding cytoplasm [7,11–17], and the nucleus has therefore been considered the rate-limiting organelle for cell migration through matrix pores [15,18,19]. In agreement, highly invasive cells, such as cancer cells – but also cells of the immune system – often feature soft and flexible nuclei [20–22]. Understanding the molecular mechanisms regulating nuclear mechanics is therefore important for better understanding tissue invasion and tumor metastasis [7,15,18].

The nuclear lamina underlying the inner nuclear membrane provides a dominant contribution to the mechanics of the interphase nucleus. Lamins, a family of intermediate filament proteins, are an integral component of the nuclear lamina and important

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regulators of nuclear stiffness [14,15,23–25]. They include the lamin A/C subtypes and connect the nucleus to the cytoskeleton via the LINC (Linker of Nucleus and Cytoskeleton) complex which in turn can interact with actin-, vimentin-, and tubulin-based filaments [7,13–15,26]. If this linkage is disturbed in cells lacking lamin A/C, cells often have softer and misshaped nuclei and increased nuclear fragility leading to decreased cell viability [7,13–15,17,24,27,28]. Diverse mutations in the *lmna* gene or in lamin-binding proteins cause a group of human diseases jointly referred to as laminopathies, including Emery-Dreifuss muscular dystrophy and premature aging syndromes [11,12,14,17,24,29,30], underlining the importance of lamin A/C for proper cell mechanics and behavior.

Given the structural and chemical complexity of cell–matrix interactions, it is often difficult to obtain experimental access and study these processes *in vivo*. Therefore, artificial 3D systems mimicking the native cell surrounding are highly desirable tools for studying and analyzing the behavior of single cells in defined microenvironments. A number of approaches for designing and fabricating such artificial non-cytotoxic 3D scaffolds have been developed [31]. Direct Laser Writing (DLW) is a particularly versatile approach for building highly-defined and freely-scalable 3D structures for cell culture purposes [31–33]. DLW employs two-photon polymerization (2PP) to construct freeform 3D polymer structures attached to a carrier substrate [31–35]. Briefly, in this single-step technique a femto-second laser beam is focused into a photosensitive material using a high numerical aperture lens. In the focal volume of the laser beam simultaneous absorption of two photons leads to excitation of photosensitive molecules. This leads to a localized chemical polymerization event that is confined to the focal volume of the laser due to the non-linearity of the 2PP process [31–35].

In this study, we have used DLW to fabricate 3D cell culture scaffolds with different pore sizes on glass substrates or on a microporous membrane. 3D cell culture scaffolds on microporous membranes can be combined with an external chemoattractant gradient (fetal calf serum) which is applied across the microporous membrane. We hypothesize that 3D scaffolds on microporous membranes can be used to study both biochemical aspects (chemotaxis) and biophysical parameters (nuclear mechanics) regulating scaffold invasion of specific cell types in a single experimental setup.

2. Material and methods

2.1. Cell culture

A549 cells (human lung carcinoma cells) were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and cultured in F-12K medium (Gibco). Wildtype mouse embryonic fibroblasts (MEF WT) and *lmna*$^{-/-}$ MEFs (MEF *lmna* KO) were a generous gift from Colin Stewart (Astar, Singapore) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco). Cell culture media were supplemented with 10% fetal calf serum (FCS, HyClone). Cells were cultivated in an incubator at 37 °C with 5% CO_2 in a humidified environment and passaged three times a week.

2.2. Transfection

A549 cells (3×10^5) were seeded in a culture flask 24 h prior of transfection. Lipofectamine 2000 (25 μl, Invitrogen) and 6.25 μl of a 10 μM lamin A/C siRNA (Ambion) or GFP-labeled control siRNA (Qiagen) solution were added separately to vials containing 600 μl F-12K medium and then incubated for 5 min. Both solutions were mixed and incubated for further 20 min. After the cells were rinsed with phosphate buffered saline (PBS, Gibco), siRNA solution and F-12K medium were added, resulting in a total volume of 5 ml. After 24 h, cells were rinsed first with PBS and then with F-12K medium containing 10% FCS. Cells were used for experiments after another 2 days of incubation.

2.3. Nuclear stiffness measurements

Atomic force microscopy (AFM) cell elasticity measurements were performed using a NanoWizard II AFM (JPK Instruments, Germany) mounted on top of an inverted optical microscope (Carl Zeiss Axio Observer A1) and a designated Petri Dish holder. Cells were cultured for 24 h in glass bottom dishes, rinsed with PBS and adapted to CO_2-independent medium (Invitrogen, Germany) for 1 h at 37 °C and

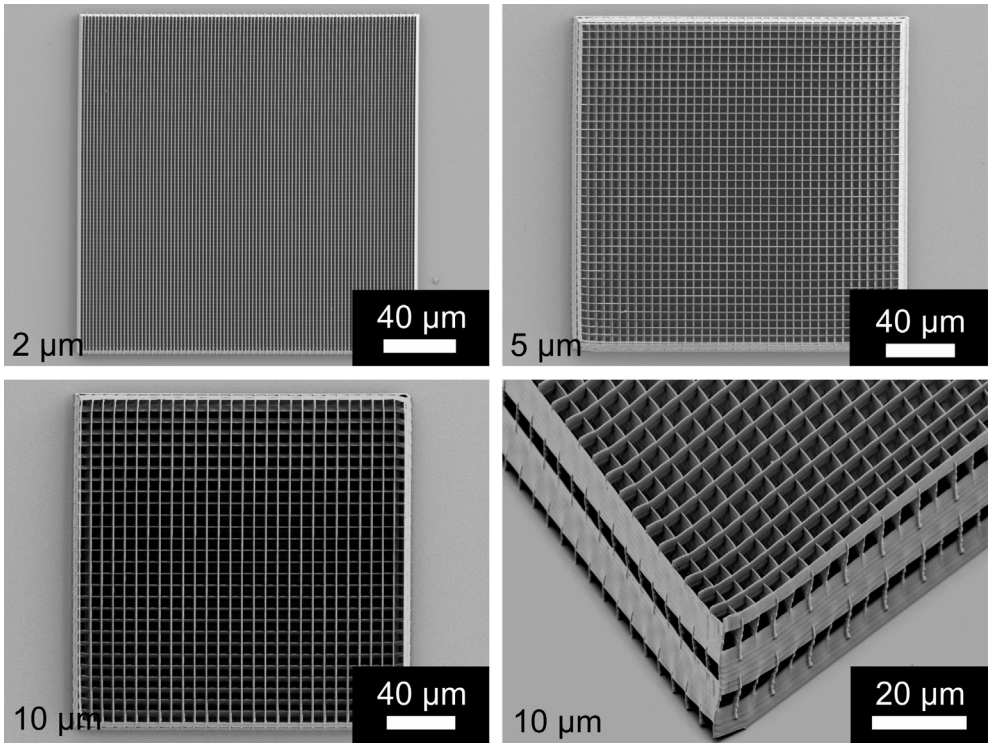

Fig. 1. Microporous 3D cell culture scaffolds produced by direct laser writing (DLW). Sacnning electron microscopy (SEM) images of 3D pentaerythritol tetraacrylate (PETTA) scaffolds with mesh sizes of 2 μm, 5 μm, and 10 μm fabricated by DLW.