



## Shape-dependent cell migration and focal adhesion organization on suspended and aligned nanofiber scaffolds



Kevin Sheets<sup>a</sup>, Stephen Wunsch<sup>b</sup>, Colin Ng<sup>c</sup>, Amrinder S. Nain<sup>a,c,d,\*</sup>

<sup>a</sup> School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA, USA

<sup>b</sup> Department of Biology, Virginia Tech, Blacksburg, VA, USA

<sup>c</sup> Department of Mechanical Engineering, Virginia Tech, Blacksburg, VA, USA

<sup>d</sup> Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA, USA

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### ABSTRACT

In the body, cells dynamically respond to chemical and mechanical cues from the extracellular matrix (ECM), yet precise mechanisms by which biophysical parameters (stiffness, topography and alignment) affect cell behavior remain unclear. Here, highly aligned and suspended multilayer polystyrene (PS) nanofiber scaffolds are used to study biophysical influences on focal adhesion complex (FAC) arrangement and associated migration behavior of mouse C2C12 cells arranged in specific shapes: spindle, parallel and polygonal. Furthermore, the role of cytoskeletal-altering drugs including blebbistatin, nocodazole and cytochalasin-D on FAC formation and migratory behavior is investigated. For the first time, this work reports that cells on suspended fiber networks, including cells with administered drugs, elongated along the fiber axes and developed longer ( $\sim 4\times$ ) and more concentrated FAC clusters compared to cells on flat PS control substrates. Additionally, substrate designs which topographically restrict sites of cell attachment and align adhesions were found to promote higher migration speeds (spindle:  $52 \mu\text{m h}^{-1}$ , parallel:  $39 \mu\text{m h}^{-1}$ , polygonal:  $25 \mu\text{m h}^{-1}$ , flat:  $32 \mu\text{m h}^{-1}$ ). This work demonstrates that suspended fiber topography-induced concentration of FACs along fiber axes generates increased migration potential as opposed to flat surfaces, which diffuse and randomly orient adhesions.

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### 1. Introduction

Cell migration is fundamental to a number of biological processes including tissue growth and regeneration, wound healing, the immune response and cancer metastasis [1,2]. In the body, cells migrate by attaching to and interacting with their immediate microenvironment known as the extracellular matrix (ECM), a fibrous mesh primarily composed of fibrils with diameters of 30–70 nm, which can bundle into 200 nm–1  $\mu\text{m}$  fibers [3,4]. The ECM mostly consists of nanofibrous collagen, elastin, fibronectin, laminin and proteoglycans, but the exact composition and alignment vary among different tissue microenvironments [5,6]. This network of micro/nanofibers serves as a scaffold that provides cells with mechanical and chemical cues which, along with external stimuli, dictate individual cell behavior. The combination of these influences can cause significant changes to cell shape, spreading, migration and differentiation, yet it remains unclear how biophysical cues alone affect ultimate cell function [7,8].

Since cells naturally interact with the ECM, an ideal platform by which migratory events can be studied effectively would use substrates which mimic this arrangement in a repeatable manner. Attempts to isolate substrate effects on cell behavior include both two-dimensional (2-D) and three-dimensional (3-D) approaches. Many 2-D gels can be cross-linked at different densities to produce substrates of varying elastic modulus, but the resulting flat surface prevents cells from either forming adhesions out of plane or having to navigate obstacles [9–11]. To compensate, cells are often subject to a 3-D surface or encapsulated within a gel, which makes single-cell studies increasingly difficult [12–16]. Fiber manufacturing platforms such as electrospinning allow some control of substrate porosity, but these techniques often fail to produce substrates with a high enough degree of repeatability required for single-cell analysis [17–19].

Despite lacking an ideal ECM-mimicking substrate, many recent studies have focused on elucidating the role of biophysical cues in cell performance by creating durotactic environments specifically designed to direct cell shape and associated cell motion [20–22]. The shape that a cell takes on as a result of physical cues has proven very useful in understanding its mechanical state [23,24]. Living cells actively generate forces in their contractile actomyosin

\* Corresponding author at: School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA, USA. Tel.: +1 540 231 6036.

E-mail address: [nain@vt.edu](mailto:nain@vt.edu) (A.S. Nain).

cytoskeleton [25,26]. When activated, trans-membrane receptors called integrins sense and transmit externally applied stresses to the cytoskeleton [27]. Integrins mechanically couple the cytoskeleton to the ECM through large, multi-protein domains known as focal adhesion complexes (FACs), which contain most notably paxillin, vinculin and talin in addition to many other components [28–30]. FACs are thought to mature in accordance with substrate rigidity, with larger FACs indicative of increased contractility and decreased migration on flat substrates, and are therefore useful in understanding cell–substrate interactions [31,32]. The main contribution to cytoskeletal mechanical integrity is thought to arise from filamentous actin (F-actin), microtubules and intermediate filaments. Of the three, F-actin is perhaps most important as it constitutes 1–10% of all proteins in the cell, responds rapidly to external forces, and is instrumental in the formation of leading-edge protrusions during cell migration [33–35]. Individual F-actin elements combine together in bundles to form tertiary filamentous structures called stress fibers, which play a dominant role in cytoskeletal dynamics of migrating cells on 2-D surfaces. Migration is central to the functionality of cells in developmental stages, during wound healing and for combating infection, and is implicated in cancer metastasis and angiogenesis. Thus, relating the mechanical response of a cell to external stimuli and correlating the response with the sub-cellular organelle functionality is important not only for advancing fundamental understanding of overall cell behavior, but also for the eventual treatment of disease.

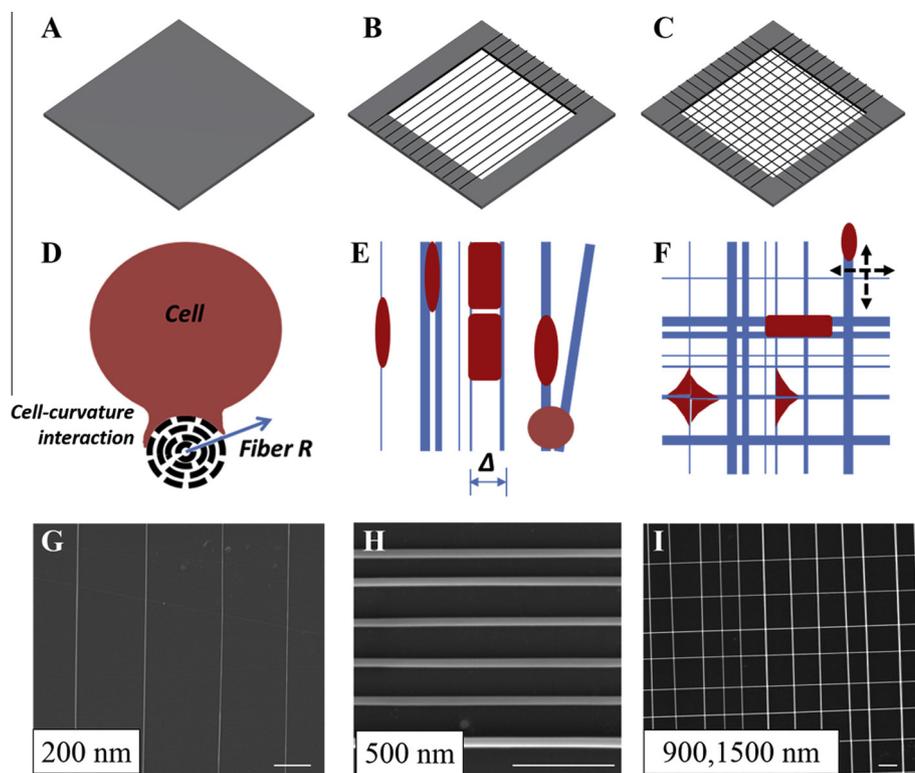
In this work, the non-electrospinning STEP (spinneret-based tunable engineered parameters) technique is used to fabricate suspended fibrous scaffolds specifically designed to position cells in repeatable shape configurations, and to relate migration tendencies of these shapes to FAC formation and maturation. Scaffold geometrical parameters such as fiber diameter, spacing and

orientation are altered to present cells with suspended fiber topographies which restrict FAC formation along the fiber axes. As cells attach to the fibers and take on these repeatable shapes, changes in migration speed are observed and linked to FAC development. Furthermore, cytoskeleton-altering drugs which knockdown or inhibit F-actin, microtubules and myosin II are introduced to further probe cell–fiber effects compared to traditionally studied flat substrates. To the best of our knowledge, this is the first study to elucidate cytoskeletal drug effects on FAC formation and associated cell migration on suspended 3-D fibrous substrates which resemble native ECM.

## 2. Materials and methods

### 2.1. STEP scaffold manufacturing

STEP is a pseudo dry spinning process based on polymer molecular chain entanglement density. Polystyrene ( $M_w$ : 2E6 g mol<sup>-1</sup> dissolved in xylene to various wt.%) scaffolds were created using the previously reported STEP platform, which deposits high-aspect ratio micro/nanofibers in highly aligned arrays [36–39]. Briefly, polymer solution is pumped through a spinneret and collected as aligned fiber arrays on substrates of varying materials, thickness, shape and dimensions. In addition to using flat polystyrene control substrates without fibers, aligned polystyrene micro/nanofibers were spun onto two different scaffold geometries named according to their layering style: suspended single (SS) and suspended double (SD) (Fig. 1). Suspended fibers can be viewed as a 3-D environment from the cell's perspective as they attach around the fiber curvature compared to 2-D attachment on flat substrates (Supplementary information 1).



**Fig. 1.** (A–C) Substrate designs used in STEP manufacturing include flat PS, suspended single layered fibers (SS) and suspended double layered, intersecting fibers (SD). (D) Cross-section view of a typical cell interacting with a suspended fiber. Of particular interest is how a cell's migration dynamics change when attached to fibrous substrates of varying sub-cellular radii (shown underneath the cell cartoon). (E) Typical cell shapes (red) when attached to SS fibers (blue). (F) Typical cell shapes when attached to SD fibers. (G–H) STEP capabilities demonstrating control of fiber diameter and spacing, and (I) deposition in multiple layers with ordered spacing and alignment. Scale bar = 5  $\mu$ m.

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