



## Review

Multiscale relationships between fibronectin structure and functional properties <sup>☆</sup>M.J. Bradshaw<sup>a</sup>, M.L. Smith<sup>b,\*</sup><sup>a</sup> Department of Mechanical Engineering, Boston University, 44 Cummington St., ERB 502, Boston, MA 02215, USA<sup>b</sup> Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA

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## ABSTRACT

Cell behavior is tightly coupled to the properties of the extracellular matrix (ECM) to which they attach. Fibronectin (Fn) forms a supermolecular, fibrillar component of the ECM that is prominent during development, wound healing and the progression of numerous diseases. This indicates that Fn has an important function in controlling cell behavior during dynamic events in vivo. The multiscale architecture of Fn molecules assembled into these fibers determines the ligand density of cell adhesion sites on the surface of the Fn fiber, Fn fiber porosity for cell signaling molecules such as growth factors, the mechanical stiffness of the Fn matrix and the adhesivity of Fn for its numerous soluble ligands. These parameters are altered by mechanical strain applied to the ECM. Recent efforts have attempted to link the molecular properties of Fn with bulk properties of Fn matrix fibers. Studies of isolated Fn fibers have helped to characterize the fiber's material properties and, in combination with models of Fn molecular behavior in the fibers, have begun to provide insights into the Fn molecular arrangement and intermolecular adhesions within the fibers. A review of these studies allows the development of an understanding of the mechanobiological functions of Fn.

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## 1. Introduction

Mechanobiology encompasses a diverse field aiming to determine how physical properties are transduced by cells into changes in behavior or phenotype. In this context, cells sense both the passive physical properties of their microenvironment as well as changes due to externally applied stress [1,2]. One challenge in studying mechanobiology is that physical properties are often intertwined, and it is thus beneficial to categorize different aspects of the microenvironment to assess their importance in the regulation of cell function. An oft-used approach is to lump properties according to biochemistry, mechanics and topography. Biochemistry includes the structure/function relationship that regulates the specificity of interaction. Cells recognize type I collagen vs. fibronectin (Fn) owing to the specificity of different integrins that bind each extracellular matrix (ECM) structure. ECM structures such as Fn bind and present growth factors to cells, which are recognized through specific growth factor receptors, and thus coordinate cell behavior. Mechanical properties include the viscoelastic properties of the microenvironment as well as imposed mechanical stress on cells. This is a broad area of mechanobiology, since, for example,

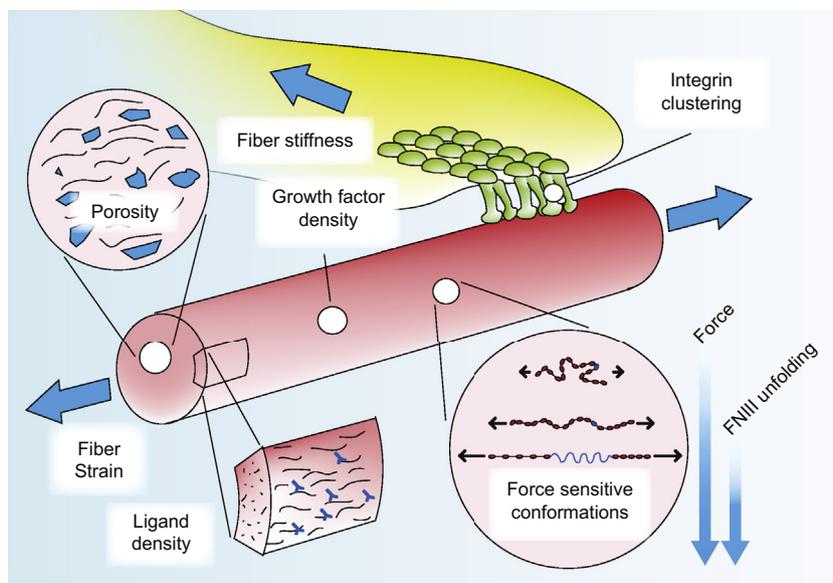
endothelial cells and pulmonary epithelial cells have dissimilar mechanical loading environments, i.e., shear stress and tissue stretch, respectively, and, in other contexts such as cancer, tissue rigidity appears to contribute to disease progression [3,4]. Finally, topographical cues result from a wide range of properties, including dimensionality and the spacing and density of adhesion ligands. Other microenvironmental factors such as matrix porosity and cell density can affect cell shape, which itself can control even the most basic of cell behaviors [5–8]. Fig. 1 illustrates these properties in the context of Fn matrix fibers, and Table 1 summarizes several properties of Fn that are sensed by cells.

In vivo, the local microenvironment is composed of other cells and/or an ECM, and, for this reason, a deep understanding of mechanobiology requires that the physical properties of both cells and ECM are first characterized. The present review highlights exciting recent advances in the understanding of the physical properties and hence the mechanobiological functions of Fn, a prevalent component of the ECM during development and progression of diseases such as cancer and atherosclerosis. First, it is important to recognize that Fn is an extremely well-studied protein, and a PubMed query using the search term “fibronectin” yields more than 33,000 hits as of 2013. Indeed, a number of outstanding reviews have already been written on a variety of topics related to Fn [30], including listing Fn binding sites for cells and cell signaling molecules [31,32] and describing the process by which cells

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**Fig. 1.** Several physical properties of Fn are believed to influence cell behavior, all of which vary with the tension applied to the fiber. The fiber stiffness is a purely physical property sensed by cells through attachments to the fiber through which the cells apply force. Fn fibers may act as a reservoir for growth factors, which must diffuse through the porous Fn gel. The number and spatial distribution of accessible ligands may be sensed through mechanisms such as integrin clustering. Fn has many cryptic binding sites that are activated or deactivated by changes in the conformation of the molecule. These conformations are altered by force applied to the molecule. Table 1 provides additional information pertaining to these properties and relevant literature references.

**Table 1**

Properties of the Fn ECM alter the behavior of attached cells through the sensation of strain-sensitive ECM properties: those properties are described and literature references to examples of their biological function are provided.

| Mechanism                              | Description  | References |
|--|--|------------|
| Porosity                               | Determines what size molecules are able to get into the fiber. Also partly determines how transport within the fiber occurs  | [9]        |
| Growth factors                         | Growth factors are stored in the ECM. Attachment to the ECM also improves their activity   | [10–16]    |
| Integrin clustering/<br>ligand density | There are multiple integrin binding sites on Fn fibers. Their spatial arrangement and density could regulate cell attachment   | [17–21]    |
| Conformational switch                  | Unfolding occurs in portions of Fn matrix molecules when they are placed under tension from cell traction forces. These unfolded regions display new binding sites previously hidden within the molecular fold. These sites could act as force transducers | [22–27]    |
| ECM stiffness                          | The stiffness of Fn matrix fibers to which a cell is attached may have major effects on the cell behavior  | [28,29]    |

produce Fn matrix fibers [33–35], and therefore these topics will not be covered in depth here. Despite the breadth of studies on Fn, there are still a number of fundamental properties of Fn matrix that have only recently begun to be investigated or remain unknown.

## 2. Fibronectin structure

Fn is a dimeric protein made of two ~250 kDa subunits, which are linked by a pair of disulfide bonds at their c-terminal ends [31]. The molecule is composed of domains of type I, II and III, which fold into globular modules connected in series. This connection of modules in series provides Fn with a very long contour length of 120–160 nm [36], and thus Fn's binding sites are distributed along its length. Modules of type I (FnI) and II (FnII) each contain

two disulfide bonds [31] that structurally reinforce these modules. Domains of type III (FnIII) do not have any internal disulfide bonds, and the absence of disulfide bonds and the free energy of denaturation led to the hypothesis that these domains could be unraveled by mechanical force [37]. From a structural perspective, the potential for unraveling or partial unfolding could affect Fn function through a variety of mechanisms. First, unfolding could expose binding sites that were previously hidden within the folded structure, known as cryptic binding sites. Second, unfolding could interrupt binding sites present in the equilibrium structure, thus turning binding sites off. Loss of quaternary structure could open up binding sites that were sterically blocked, leading to an alteration of function without loss of secondary/tertiary structure. Finally, partial unfolding or extension of the molecule could extend Fn such that binding partners that utilize multiple binding sites are limited in the extent of coordination. This would lower avidity without altering the affinity of each individual binding domain, thus reducing binding.

## 3. Fibronectin biosynthesis

Fn is encoded by a single gene, and the pre-mRNA is alternatively spliced to create a large number of splice variants [31]. These splice variants include the omission of 1 or 2 FnIII domains known as EDA and EDB. When present, EDA occupies the space between FnIII<sub>11</sub> and FnIII<sub>12</sub>, while EDB resides between FnIII<sub>7</sub> and FnIII<sub>8</sub>. In humans, there are five variations of different molecular length within the V, or variable in length, region between FnIII<sub>14</sub> and FnIII<sub>15</sub> [31]. Plasma Fn, secreted by hepatocytes and circulating in the bloodstream at a concentration of 300 µg ml<sup>-1</sup> [38], does not generally include the EDA and EDB domains, but may include the V region. Fn secreted by cells at the site of matrix assembly, known as cellular Fn, includes a larger number of isoforms and may include both EDA and EDB [31].

The functional form of Fn in vivo is in its fibrillar state. Thus, Fn molecules must be assembled into supermolecular fibers that range in diameter from 10 nm to micrometers in size, with lengths of tens of micrometers. These fibers form an interconnected network that

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