Influence of nucleus deformability on cell entry into cylindrical structures

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Abstract The mechanical properties of cell nuclei have been demonstrated to play a fundamental role in cell movement across extracellular networks and micro-channels. In this work, we focus on a mathematical description of a cell entering a cylindrical channel composed of extracellular matrix. An energetic approach is derived in order to obtain a necessary condition for which cells enter cylindrical structures. The nucleus of the cell is treated either (i) as an elastic membrane surrounding a liquid droplet or (ii) as an incompressible elastic material with Neo-Hookean constitutive equation. The results obtained highlight the importance of the interplay between mechanical deformability of the nucleus and the capability of the cell to establish adhesive bonds and generate active forces in the cytoskeleton due to myosin action.

Keywords Nucleus deformability · Cell migration · Micro-channel · Cell mechanical properties · Integrin-mediated migration model

1 Introduction

Cell migration inside extracellular matrix networks plays a critical role in many physiological and pathological processes. For instance, in wound healing, the deposition of extracellular matrix (ECM) and the migration of cells through it contribute to repair both epithelial layers and connective tissues, whereas in immune surveillance and inflammation, leukocytes actively migrate towards the site of infection (Friedl and Weigelin 2008). On the other hand, in pathological conditions, cell migration is involved, for example, in chronic inflammatory diseases and in cancer cell invasion and metastasis formation (Sahai 2007).

Moreover, with tissue engineering advent, the process of cell migration is also exploited in biomedical applications for the regeneration of various tissues, both in vivo and in vitro (Capito and Spector 2003; Yannas et al. 1989).

From the biological point of view, an increasing number of experimental works has been designed in order to determine cell properties and functions that are involved in the dynamics of motion inside the extracellular microenvironment and the contribution of this complex network of structural fibrous proteins on the overall process (see, for instance, Guck et al. 2010; Lautenschläger et al. 2009; Rolli et al. 2010; Wolf et al. 2007). In particular, the key factors for cell migration on flat substrates are the dynamic adhesion of cells on it via the expression of adhesive molecules (mainly integrins) and the generation of the force necessary for propulsion by contraction of cytoskeletal elements (Friedl and Wolf 2009). These are also the basic “ingredients” in the process of migration inside three-dimensional (3D) porous environments. However, in this case, cells require steering their way throughout steric obstacles. This process can be supported by the production of proteolytic enzymes (e.g. Matrix Metalloproteinases, MMPs) able to degrade matrix components in order to open gaps for cell movement (Friedl and Brocker 2000; Friedl and Wolf 2003; Sabeh et al. 2009; Wolf and Friedl 2011). The migratory and invasive process in three-dimensional environments is generally associated with both significant cell deformation and cytoskeletal force generation while passing through constricted openings of the ECM (Rolli et al. 2010; Wolf et al. 2007). The cell body basically consists of the cytoplasm and the nucleus. The cytoplasmatic region is highly
Fig. 1 Nuclear deformation during cell migration: biological experiments and schematic representation of the process. a Invasion of primary human melanoma cells in stroma, showing heterogeneously shaped, elongated and cigar-shaped deformed nuclei (with permission from Friedl et al. 2011). b Biological sketch of the process of a cell migrating through 3D matrix of fibres. Dots denote focal contacts, pink lines stand for ECM fibres. c Schematic representation of the geometry considered in the model: the cytosol (light blue) can freely move into one of the cylindrical channels composed of ECM bundles, whereas the nucleus stands on the back and progressively deforms in order to enter the channel adaptable to morphological changes, and it can adjust to virtually any shape (Friedl et al. 2011). On the other hand, the nucleus is 5–10 times stiffer than the surrounding cytoskeleton, and it can resist to changes in shape (Caille et al. 2002; Friedl et al. 2011). Thus, nucleus deformability is a limiting factor in the process of cell migration (Friedl et al. 2011; Wolf et al. 2007).

Cell nuclei are exposed to a variety of mechanical stresses and deformations (Kumar and Weaver 2009), especially when the proteolytic machinery is inhibited or during migration inside artificial rigid scaffolds. Cellular and nuclear deformations require substantial reorganization of the cytoskeleton and compression of the keratin envelope of the nuclear region in order to acquire an elongated configuration (see Fig. 1a). Indeed, it has been observed that, inside ECM channels, nucleus shape and keratin network structure strongly deviate from the normal spatial distribution in the undeformed cell (Rolli et al. 2010).

These biological findings highlight that the dimensionality of the environment strongly affects cell migratory capabilities (Harley et al. 2008; Kuntz and Saltzman 1997) and that the deformability of the cell, and in particular of the nucleus, is crucial for cell migration in 3D structures.

The first efforts in describing mechanical behaviour of living cells were aimed at understanding the response to mechanical stress by cells living in the vascular system (e.g., red and white blood cells (Skalak 1973)). In order to measure the mechanical properties of cells, they must be deformed in some way by a known force or stress, and the corresponding deformation must be measured.

In the last years, several tests have been developed for this purpose, based on mechanical instruments such as atomic force microscope (AFM), optical traps (laser tweezers), microcompression method and micropipette suction (Hochmuth 1993, 2000; Liu 2006). However, most of the works in the literature do not distinguish among the contributions of the different cellular structures and measure a general cellular stiffness, even though it is known that distinct elements can present really different mechanical properties and, in particular, at least the nucleus contribution should be distinguished from the cytoplasmic response during deformation (Caille et al. 2002). Moreover, despite the wide range of technological instruments available, the description of the mechanical properties of cells and cell nuclei is still at a primitive stage, and a constitutive theory able to describe their behaviour is still missing.

Even though the scientific community is becoming aware of the importance of mechanical properties of cells and cell nuclei in their process of migration inside porous structures, poor investigations have been carried on from the mathematical modelling point of view and mechanical information is generally neglected in the description of cell movements.

Nowadays, in macroscopic models, the movement of a population of cells inside a region containing extracellular matrix is described by balance laws derived by continuum mechanics, in which the mechanical properties of cell nuclei are not considered.

Even when we move towards the length scale at which discrete models are used, the mechanical properties of cells are poorly considered, unless we move towards really detailed models of the cells. One of them is the tensegrity model (Ingber 1993, 2003), in which loads are supported by struts in compression and cables in tension. Clearly, this description is closer to reality, but the high complexity of this model makes it difficult to be used in simulations with a large number of cells and to be up-scaled to the description of macroscopic behaviours.

Some first efforts to include cells mechanical properties inside the description of cell movement on 2D and inside 3D substrates have been done by Scianna et al. (2013), Scianna and Preziosi (2013) using Cellular Potts Models (CPM), which allow intuitive representation of cells and their mechanical properties, without requiring too expensive computations.
The introduction of microscopic mechanical properties of cells and subcellular structures into continuum macroscopic equations are of fundamental importance in order to make a step towards a more comprehensive representation of cells and tissues. As a first step in this complex problem, we study how the nucleus deformability can influence the process of a cell entering a 3D extracellular structure, using a continuum description of the cell nucleus. Even though, in vivo, fibre structures and bundles are arranged into really complex networks of strongly varying local densities (Wolf et al. 2009), that create pores and gaps, we simplify the problem, considering the ECM structured in parallel cylindrical channels composed of fibres and bundles that provide directional guidance cues to cells (see Fig. 1c). This is of course a strong assumption of the far more complex real structure of the extracellular environment, but it can be a good approximation for regular scaffolds used in tissue engineering, for microchannel setup used to test cell deformability and migration capacity, for packed collagen bundles consisting of multiple aligned fibres, for myofibres and for nerve strands (Wolf and Friedl 2011). Moreover, it helps to make a first step towards the description of the real phenomenon.

This paper is organized as follows: In Sect. 2, we review micropipette experiments and the related classical aspiration criteria, commenting the difficulties encountered in applying them to the description of a cell entering an ECM channel. It is shown that these models cannot account for the boundedness of cells and, thus, lead to some unrealistic results (see “Appendix 1”). A simple mathematical model for active forces, required to accomplish the process, is analysed in Sect. 3. Here, we focus only on integrin-dependent migration (Wolf et al. 2007), in which the process of cell adhesion with the substrate is fundamental in order to activate the actomyosin contraction necessary for nucleus deformation and cell movement along the track.

In this work, we do not claim to develop a comprehensive model of the complex meshwork surrounding the cell nucleus and of the mechanism of active force generation. Indeed, cells are really complex assembly and, even when the mechanical properties of single elements are biologically known, the cell global behaviour may be different from that of one of its isolated components (Caille et al. 2002). Further investigations are thus required in order to achieve a reliable model of cell active force generation.

Here, we start from the biological observation that the traction force is related to focal adhesion (Versaevel et al. 2012) and we make different hypotheses on the active traction force (acting on the nucleus), generated after the formation of a single bond. In particular, we point out two possible representations of this force (linear vs. constant in space) and we make some considerations on the extension of the adhesive area (boundedness assumption). In Sect. 4, we propose a mathematical model, based on an energetic approach, able to describe the deformation of a spherical finite elastic structure (representing the nucleus) into an elongated deformed one that can move inside the channel. Two different representations for the deformed nuclear configuration are implemented (ellipsoidal vs. cigar-shaped). The nucleus is mechanically assimilated either to an elastic membrane (Sect. 4.1) or to an elastic solid (Sect. 4.2). In both cases, we assume that no nuclear loss is experienced by the cell during deformation. The computational findings are reported and discussed in Sect. 5. Results are presented in terms of dimensionless parameters that describe the interplay between mechanical and active properties, where the term “active properties” refers to cell adhesive and contractile capabilities.

2 Mathematical model of micropipette aspiration

A cell can be schematically represented as consisting of two main compartments, the cytoplasm and the nucleus, both surrounded by membranes. The cytoplasm holds all cell’s internal sub-structures (except for the nucleus) immersed in what is called cytosol. The cytosol, which fills much of the volume of the cell, is composed by a complex mixture of cytoskeleton filaments, dissolved macro-molecules and water. The cell cytoskeleton is a network of long filaments interacting with motor proteins, which use the energy deriving from the hydrolysis of ATP (adenosine triphosphate) to produce active forces and deform the network and, consequently, drag the cell nucleus (Hawkins et al. 2009). If the cell has to pass through dense fibre networks, with gaps smaller than the cell nucleus, the nucleus has to deform. The nucleus is less deformable than the cytoplasm (Caille et al. 2002; Friedl et al. 2011), and its deformability is mainly regulated by both chromatin structure, and lamin intermediate filaments (Friedl et al. 2011; Gerlitz and Bustin 2011).

When migrating inside a thick 3D fibrous environment made of extracellular matrix, with typical channel size smaller than the cellular diameter, cells need to deform both their cellular body and their nucleus. Being the nucleus the stiffest organelle (Caille et al. 2002; Friedl et al. 2011), the nuclear deformability strongly contributes to the migration efficiency of a cell in 3D environments, whereas it is much easier for the cytosol (and the embedded organelles) to intensively change its shape.

In order to describe the above complex environment, we simplify the geometry by considering the entry of a cell into a cylindrical microchannel. In this respect, micropipette aspiration is one of the most common way to study the mechanical behaviour of living cells, and it can help understanding the process of a cell entering a functionalized channel. In the typical experiment, a cell is aspirated into a small glass tube by applying a suction pressure. The leading edge of its surface is tracked with light microscopy. It is observed that,
if the suction pressure is sufficiently high, both soft cells (e.g., neutrophils, which normally transmigrate across small pores) and more rigid cells (e.g., chondrocytes and endothelial cells) completely enter pipettes, within a certain range of calibres (see Hochmuth 2000 for a review). In both cases, the response to an aspiration pressure is similar until a hemispherical projection is formed inside the pipette. Beyond that point, for cells behaving like a liquid surrounded by a membrane, a further increase in the suction pressure can cause the complete entry of the cell into the channel (Evans and Yeung 1989). On the other hand, when cells behaving like a solid are aspirated, they do not flow into the micropipette when the aspiration length exceeds the pipette radius. Instead, the surface extends until a new equilibrium position is reached (Jones et al. 1999; Theret et al. 1988). Because of the small suction pressures relative to the osmotic pressure of isotonic saline solution in which cells are positioned, in all these experiments, cells usually deform at constant volume (Hochmuth 2000).

Some simple continuum models, treating the cell either as a liquid droplet surrounded by an elastic cortical shell (Yeung and Evans 1989), or as a homogeneous elastic membrane (Chien et al. 1978), or as a solid (Theret et al. 1988), have been formulated in order to fit experimental data. Even though these models do not consider the high heterogeneity in cell composition, they surprisingly make good predictions of the cell deformation response to known suction forces produced by the pipette and they are still used today in the biomechanical community.

In Evans’ model (Yeung and Evans 1989), cells are described as passive viscous liquid droplets encapsulated by a distinct cortical layer, with cortical tension, \( T_c \). The equilibrium condition comes directly from Laplace law applied to the suction of a cell until a hemispherical projection is formed inside the pipette. Calling \( L_p \) the aspirated length and \( R_p \) the pipette radius, the critical suction pressure drop \( \Delta P_c \) is obtained for \( L_p / R_p = 1 \), when the following relation holds

\[
\frac{\Delta P_c R_p}{T_c} = 2 \left( 1 - \frac{R_p}{R_c} \right),
\]

where \( R_c \) is the radius of the cell outside the pipette (when \( L_p / R_p = 1 \)). The cortical tension creates a threshold pressure drop below which the cell will not enter the pipette and above which cells can flow into it. Moreover, Yeung and Evans (1989) observed that the rate at which a cell flows into a pipette is almost constant, with only a small nonlinearity over time until the cell completely enters the pipette.

When the cell has totally entered the pipette, all the microscopic “ruffles” and “folds” have been pulled smooth. However, Yeung and Evans (1989) observed that there exists a lower limit below which cells cannot enter the pipette, which for the specific cells used in their biological experiments (granulocytes) corresponds to a calibre of 2.7 \( \mu m \) (Evans and Kukan 1984; Evans and Yeung 1989).

For what concerns cells behaving like a solid, many studies have been done on human red blood cells. The deformation of such anucleate cells has been studied under a constant area assumption (Chien et al. 1978; Evans 1973; Evans and Waugh 1976; Waugh and Evans 1979), to derive the suction pressure \( \Delta P \) needed to aspire a portion of cell of length \( L_p \) inside a cylindrical channel of radius \( R_p \). This is given by the following relation (that holds for \( L_p > R_p \))

\[
\frac{\Delta P R_p}{\gamma} = 2 \frac{L_p}{R_p} \log \left( 1 + 2 \frac{L_p}{R_p} \right),
\]

where \( \gamma \) is the shear elastic modulus of the membrane.

Equation (2.2) is obtained as the stationary condition of the dynamic relation

\[
\frac{\Delta P R_p}{\gamma} = 2 \frac{L_p}{R_p} - 1 + \frac{2}{\gamma} \frac{L_p}{R_p} + \frac{4}{\gamma} \frac{L_p}{R_p},
\]

where a viscoelastic stress-strain relation is assumed for the membrane, with viscosity \( \eta \).

It has been experimentally observed that, when \( \frac{L_p}{R_p} > 1 \), the relation between \( \frac{\Delta P R_p}{\gamma} \) and \( \frac{L_p}{R_p} \) is almost linear, with a slope equal to 2.45. This consideration leads to the well-known Chien’s relation (Chien et al. 1978)

\[
\frac{\Delta P R_p}{\gamma} = 2.45 \frac{L_p}{R_p} \left( \frac{L_p}{R_p} > 1 \right).
\]

Finally, Theret et al. (1988) studied the entry into a channel of a cell treated as a homogeneous elastic solid, with Young’s modulus equal to \( E \). Their analysis for an infinite, homogeneous half-space drawn into a micropipette, can be summarized by the following relation

\[
\frac{\Delta P}{E} = \frac{2\pi}{3} \Phi \frac{L_p}{R_p},
\]

where \( \Phi \) is a factor linking the external and internal radius of the pipette, which is assumed to be equal to 2.1 in many papers (cf., e.g., Hochmuth 2000).

The process of a cell entering a glass tube has some similarities with the process of a cell entering a channel composed of ECM fibres. Of course in the biological process of cell migration across matrix channels, we do not have any aspiration pressure, but what makes the cell deform and enter the channel is the capability of the cell itself to form strong adhesive sites with the ECM and actively contract the cytoskeleton to deform the nucleus and pull the cell inside narrow openings (Hawkins et al. 2009). The case of interest is, thus, when the radius of the channel is smaller than the radius of the nucleus of the cell, i.e., \( R_p < R_n \). This migratory mechanism is strictly linked to the cell capability.
of establishing bonds (integrin-dependent migration), and it is the preferential way of moving of those kinds of cells that are highly adhesive (e.g., smooth muscle cells and fibroblasts). Recent works (Lämmermann et al. 2008; Renkawitz and Sixt 2010) show that integrin-independent mechanisms of motion are possible in confined environments. However, in this paper, we will refer only to adhesion-driven motion. Therefore, trying to apply the classical models recalled above to the description of a cell entering a channel, the total suction force, $\Delta P$ in Eqs. (2.2)–(2.5), should be linked to the active force generated through cytoskeleton contraction as a consequence of cell-ECM bond formation. We will give more details about these forces in the following section.

Moreover, we focus on the mechanical response of the nucleus of the cell, applying Eqs. (2.2)–(2.5) to its deformation. Even though the results obtained under these hypotheses seem promising (see “Appendix I”), we have to be aware that we are pushing the criteria beyond their limit of validity. In fact, Chien’s and Theret’s models have been obtained assuming, respectively, an infinite 2D membrane and a 3D half-space aspired inside a pipette. Moreover, the pipettes used in Chien’s biological experiments ranged from 0.3 $\mu$m up to 0.8 $\mu$m and the volumes aspirated into the pipettes were always <5% of the cell volume (Chien et al. 1978). At the same time, even though the pipettes used by Theret et al. (1988) were bigger (with an internal diameter ranging between 2 and 3 $\mu$m), the portion of the cell aspired was two-to-four times $R_p$. Therefore, both validations stay away from the complete entry of the cell. Actually, all these studies were designed in order to determine the mechanical properties of cells in the first stages of the deformation and they better apply to problems in which deformations are small (normally $L_p/R_p$ up to 5 Theret et al. 1988), whereas micropipette experiments account also for the total aspiration of the cell. These observations highlight the importance to derive simple analytical relations able to describe the process of a cell entering a cylindrical channel, overcoming the assumption of infinite dimension of the cell.

3 Active force in integrin-dependent migration

To describe cell entry into ECM channels, a fundamental step is the definition of the force, which leads to nucleus deformation and migration inside the channel.

Cell migration into 3D environments consists of different steps, cyclically reiterated by the cell (Friedl et al. 2011). In integrin-mediated locomotion, first the cell polarizes, assembling actin at the cell front into filaments, which push the plasma membrane outward and form protrusions. Then, these protrusions interact with the ECM, building strong adhesion points with the substrate, through the expression and activation of transmembrane receptors of the integrin family (Hawkins et al. 2009; Lämmermann et al. 2008). At this stage, cells possibly activate the proteolytic degradation and realignment of ECM fibres, forming tracks for cell motion. Then, actomyosin contracts the actin networks, generating local traction and the force necessary for cell movement along the track (Friedl et al. 2011) (see Fig. 1a). For most cell types, adhesion and migration are so intimately linked that regulation of substrate adhesiveness is the main factor guiding the locomotion (haptokinetic). In this type of cells, the internal cytoskeleton is strictly linked to the ECM, through transmembrane receptors (primarily integrins), on one side, and to the cellular nucleus, on the other side, through the lamin intermediate filaments forming a part of the nuclear envelope (Friedl et al. 2011; Gerlitz and Bustin 2011). This adhesiveness largely determines cell shape and nuclear deformation (Wolf et al. 2007). Moreover, it has been experimentally observed (Versaevel et al. 2012) that the traction force generated through myosin contraction depends on the focal adhesion area of the cell.

Even though recent works (Lämmermann et al. 2008; Renkawitz and Sixt 2010) show that, at least for some kinds of cells (e.g. leucocytes and some metastatic tumour cells), the migration in confined environments is sustained by integrin-independent mechanisms, in this paper, we consider only adhesion-dependent movements, in which the deformation of the nucleus during cell movement is driven by the generation of an active force in the cytoskeleton meshwork, as a consequence of bond formation, and by steric hindrance. We do not give an explicit model of active force generation, but we postulate different constitutive forms for the force exerted after the formation of a bond.

The active force related to adhesive processes can be thought of as the resultant of all forces generated by cytoskeleton contraction after single cell-ECM bond formation on the surface of adhesion. Cell-matrix adhesion is mainly mediated by integrins on the cell surface that connect ECM to the cytoskeleton, and thus, the adhesive potential of a cell is defined by its repertoire of integrin family adhesion receptors (Renkawitz and Sixt 2010). The adhesion can be modulated by the density of expressed and activated integrins, $\rho_h = N_{\text{integrin}}/S_{\text{cell}}$ (where $N_{\text{integrin}}$ is the number of integrins over the surface of the cell, $S_{\text{cell}}$), and by the density of substratum ligands (ECM adhesive sites), here represented by the ECM surface ratio, $\sigma_{\text{ECM}} = S_{\text{ECM}}/S_{\text{channel}}$. We will assume that the cytoplasm can easily penetrate inside the channel without any constriction (see Fig. 2), so that the action of cytoskeletal contraction will cause on one hand the displacement of the cytosolic region (keeping the same shape with a tip that will be modelled as a spherical cap), and on the other hand, the advancement of the nucleus, that, being at the entrance of the microchannel will deform to penetrate it. We can approximately say that the length of the region in contact with the channel wall and in front of the nucleus is approx-
immediately constant and we can assume that bonds are formed on this surface. Therefore, referring to Fig. 2 and defining 
\( S = \{(X, Y, Z) : X^2 + Y^2 = R_p^2, \bar{Z}_{\text{low}}(t) < Z < \bar{Z}_{\text{up}}(t)\} \) as the surface for which ECM-bonds are expressed, we can say that the length for which bonds are formed, \( L_b = \bar{Z}_{\text{up}}(t) - \bar{Z}_{\text{low}}(t) \), remains constant in time during cell deformation.

Accepting that the density of bonds on cell surface, \( \rho_b \), and the portion of the channel wall composed of ECM adhesive sites, \( \alpha_{\text{ECM}} \), do not depend on time, the total active force is

\[
F_{\text{active}} = \int_S \rho_b(X) \alpha_{\text{ECM}}(X) F_{\text{bond}}(X) dS, \tag{3.1}
\]

where \( F_{\text{bond}}(X) \) is the traction force exerted on the nucleus through cytoskeleton contraction, as a consequence of bond formation. Although \( \rho_b \) and \( \alpha_{\text{ECM}} \) may be generally functions of space, in the homogeneous case, Eq. (3.1) simplifies into

\[
F_{\text{active}} = \rho_b \alpha_{\text{ECM}} \int_S F_{\text{bond}}(X) dS. \tag{3.2}
\]

Considering only the \( Z \)-component of this force, we have

\[
F_{\text{active}}^Z = 2\pi \rho_b \alpha_{\text{ECM}} R_p \int_0^{L_b} F_{\text{bond}}^Z dZ. \tag{3.3}
\]

The total active force pulling the cell is therefore a function of the radius of the pipette, the density of bonds \( \rho_b \), the surface fraction of the channel composed of extracellular matrix, \( \alpha_{\text{ECM}} \), and the integral of the single bond forces over the length of contact, \( L_b \). In particular, under the assumption that bonds are formed only on the portion of the cell in front of the nucleus in contact with the channel (see Fig. 2), we have

\[
L_b = L_{\text{cell}}^0 - R_p - L_n^0 \tag{3.4}
\]

where \( L_{\text{cell}}^0 \) is the initial length of the cell inside the channel (which corresponds to the length of the deformed cytoplasm) and \( L_n^0 \) is the portion of the nucleus that can enter the pipette without any deformation. Geometrical arguments, based on volume conservation of both the cytoplasm and the nucleus, give

\[
L_{\text{cell}}^0 = R_p \left[ \frac{4 R_p^3 - R_n^3}{3 R_n^3} + \frac{1}{3} + \frac{1}{R_p} \left( L_n^0 - \frac{1}{3} L_n^0 \right) \right], \tag{3.5}
\]

\[
L_n^0 = R_n - \sqrt{R_n^2 - R_p^2}, \tag{3.6}
\]

where \( R_p \) is the radius of the cylindrical channel, \( R_n \) the radius of the nucleus, and \( R_c \) the radius of the spherical cell. Once that a proper function representing bond forces is provided, the description of active force is accomplished. In particular, we will consider the following simple forms of \( F_{\text{bond}}^Z \), which is the \( Z \)-component of the force transmitted to the nucleus when a bond is established.

3.1 Linear force

We assume that, as a consequence of a single bond formation, a force proportional to the distance between the nucleus and the site in which the bond is formed is exerted on the nucleus, through actomyosin contraction, i.e.,

\[
F_{\text{bond}}^Z = k_b Z, \tag{3.7}
\]

where \( k_b \) is the elastic constant of a virtual spring linking the bond site to the nucleus. Substituting (3.7) into (3.3), we obtain

\[
F_{\text{active}}^Z = \pi R_p \rho_b \alpha_{\text{ECM}} k_b L_b^2. \tag{3.8}
\]

This relation takes into account the biological observation that the biggest traction forces are expressed at the apical portion of the cell (Ambrosi et al. 2009; Dembo and Wang 1999; Legant et al. 2010; Peschetola et al. 2013). However, it has the disadvantage that there is no upper limit to the active force that can be exerted, which is not true. This may become important when the size of the channel is very small causing exceedingly long cell extensions (see Sect. 5).

3.2 Constant force

We assume that the traction force acting on the nucleus, generated by a single bond activation, is constant, \( F_{\text{bond}}^Z = F_b^M \), which implies that

\[
F_{\text{active}}^Z = 2\pi R_p \rho_b \alpha_{\text{ECM}} F_b^M L_b. \tag{3.9}
\]
This relation represents the fact that there is a mean traction force that can be generated and a maximum force over which bonds break (Baumgartner et al. 2000; Panorchan et al. 2006; Peschetola et al. 2013; Sun et al. 2005) and thus the cytoskeleton no more contracts, because the cell looses adhesion to the substrate.

3.3 Force over a bounded region

We consider the case in which cells are able to form bonds only over a certain area of the contact region, e.g., the apical portion of the deformed cell. Therefore, taking a constant force assumption, we have \( F_{\text{bond}} = F_M \chi_{L_M b}(Z) \), where \( L_M b \) represents the length of the maximal area of contact for which bonds are formed (adhesive region) and

\[
\chi_{L_M b}(Z) = \begin{cases} 
1 & \text{if } (L_b - L_M b) < Z < L_b \\
0 & \text{if } 0 \leq Z \leq (L_b - L_M b) \text{ or } Z \geq L_b
\end{cases}
\]

where \((\cdot)_+\) stands for the positive part of \((\cdot)\), to take into account that for protrusions smaller than \(L_M b\) all the cytoplasmic membrane participates in the adhesion process. Therefore, the total active force is represented by the following relation

\[
F_{\text{active}}^{Z} = 2\pi R_p \rho_b \sigma_{\text{ECM}} F_M L_{b,el}^*, \tag{3.10}
\]

where \(L_{b,el}^* = \min \{L_b, L_M b\}\). This relation prevents active forces from growing dramatically for \(R_p \rightarrow 0\), and it represents the fact that for very small pipette radius, the cell cannot form adhesive sites over too large areas.

A similar relation would be achieved if the interval over \(Z\) is substituted by several disconnected intervals. Also, the localization of these “adhesive sites” does not affect the final result, provided that the overall adhesive length is the same.

Analogously, it is possible to use the linear force assumption, taking \(F_{\text{bond}}^{Z} = k_b Z \chi_{L_M b}(Z)\), that leads to

\[
F_{\text{active}}^{Z} = 2\pi R_p \rho_b \sigma_{\text{ECM}} k_b L_{b,el}^* \left( L_b - \frac{1}{2} L_{b,el}^* \right), \tag{3.11}
\]

where \(L_{b,el}^* = \min \{L_b, L_M b\}\). However, with a proper re-definition of \(L_M b\) as a function of \(L_{b,el}^*\), Eq. (3.11) leads to the same results as (3.10), when \(L_b > L_M b\).

4 Energy balance models

We tackle the problem of a cell entering a cylindrical ECM channel using an energetic approach. Always working under the constant volume assumption, we develop two models to analyse the total energy required to deform the initially spherical nucleus (see Fig. 3a) into the nucleus that is totally inside a cylindrical channel. Experimental evidences (Kole et al. 2005; Versaevel et al. 2012) suggest that, when the cell is forced to cross channels of different geometries, the initially spherical nucleus deforms significantly, orienting with respect to the cell long-axis direction and taking an elongated shape. Cell elongation is associated with the formation of parallel actin bundles on either sides of the nucleus, which are responsible for the nuclear deformation and help maintaining the deformed configuration (Valerius et al. 1981). Indeed, during cell elongation, the tension in actin bundles grows and generates compressive forces acting laterally on both sides of the nucleus (Versaevel et al. 2012).

The shape of the deformed cell can be approximated either

- by a prolate ellipsoid (Versaevel et al. 2012; Wolf and Friedl 2011), with smaller axis \(R_p\) (see Fig. 3b) or
by a cigar-like shape (see Fig. 3c), with cylindrical central region of radius \( R_p \) and hemispherical caps (Friedl et al. 2011).

Both morphologies have been observed in vivo and in vitro experiments (Caille et al. 2002; Friedl et al. 2011; Versaevel et al. 2012). Of course, the morphology acquired in vivo by the nuclear shape can be more complex, especially if the geometry of the channel is not so regular.

Concerning the calculation of the energy required to deform the nucleus, we consider the two cases in which as follows:

- All the energy is spent to increase the membrane area of the nucleus, whereas the material inside is treated as an inviscid liquid that freely rearranges according to the geometry of the channel (see Sect. 4.2);
- All the energy is spent to deform the internal solid nucleus of the cell, treated as an elastic material (see Sect. 4.3).

Of course, these hypotheses are one the opposite of the other, and intermediate situations should be studied (i.e., energy of membrane plus bulk energy). We recall once again that, in both cases, the cytoplasm can freely enter the channel.

The energy required to deform the initial spherical shape will then be compared to the work done by adhesion-mediated active forces, described in Sect. 3, to make the cell enter the microchannel.

### 4.1 Work of the active traction forces

The work required to have the cell completely inside the channel should be provided by active traction forces generated inside the cell. We can express this active work as

\[
W_{\text{active}} = F_{Z,\text{active}}^\Delta L, 
\]

where \( \Delta L \) is the total displacement of the cell nucleus inside the channel, and \( F_{Z,\text{active}} \) is the resultant directed along the Z-axis of all forces exerted by cytoskeleton contraction after cell-ECM bond formation, described in Sect. 3. In the following, we will assume that \( \Delta L = L_{n,\text{fin}} - L_{n,0} \), where \( L_{n,\text{fin}} \) is the final length of the nucleus when it is totally inside the channel and it varies depending on the representation chosen for the deformation of the nucleus (see Fig. 4). Indeed, for the ellipsoidal shape, we have that \( L_{n,\text{ellips}} = 2h_e \), where

\[
h_e = \frac{R_p^3}{R_n^2} 
\]

is the longer semi-axis of the prolate ellipsoid that preserves the initial volume, whereas, considering the cigar-shaped nucleus, \( L_{n,\text{cigar}} \) is given by

\[
L_{n,\text{cigar}} = 2(h + R_p),
\]

the volume of the nucleus is treated as a liquid droplet surrounded by an elastic shell. The energy required to increase the surface area, \( \mathcal{W}_{\text{tot}}^S \), can be approximated by the following relation (Evans and Waugh 1976; Helfrich 1973)

\[
\mathcal{W}_{\text{tot}}^S = \lambda (\Delta S)^2 
\]

where \( \Delta S \) is the increase in the surface area of the cell passing from the initial spherical shape to its final conformation.

We remark that (4.4) has the same form of the term representing the energy cost for surface area increasing in Cellular Potts Models (Graner and Glazier 1992).

The increment in surface area, \( \Delta S \), can be calculated by assuming that the volume is preserved and computing the new surface area of the deformed nucleus. Using the ellipsoidal deformation assumption, the increment in the surface area is given by
\[ \Delta S_{\text{ellips}} = S_{\text{ellips}} - S_{\text{sphere}} = \]
\[ = 2\pi R_p^2 \left[ 1 + \frac{h_c}{R_pe} \sin(\theta) \right] - 4\pi R_n^2 = \]
\[ = 4\pi R_n^2 \left[ \frac{1}{2} \tilde{R}_p^2 \left( 1 + \frac{\sin(\sqrt{1 - \tilde{R}_n^2})}{\tilde{R}_n^2 \sqrt{1 - \tilde{R}_n^2}} \right) - 1 \right], \quad (4.5) \]

where \( e = \sqrt{1 - \frac{R_p^2}{h_c^2}} \) and \( h_c \) is given by (4.2) and all distances have been conveniently scaled with the nucleus radius, defining the dimensionless quantity \( \tilde{R}_p = R_p/R_n \). Therefore, \( (4\pi R_n^2)^2 \) is a function of \( \tilde{R}_p \). Actually, in the following, all the quantities with a tilde represent the corresponding length scaled with \( R_n \).

On the other hand, using the cigar-shaped deformation hypothesis we have

\[ \Delta S_{\text{cigar}} = S_{\text{cigar}} - S_{\text{sphere}} = 4\pi R_p^2 + 2\pi R_p (2h) - 4\pi R_n^2 = \]
\[ = 4\pi R_n^2 \left[ \frac{1}{3} \tilde{R}_p^2 + \frac{2}{3\tilde{R}_p} - 1 \right], \quad (4.6) \]

where the height of the cylindrical portion of the cigar, \( 2h \), is given by (4.3).

More complex formulae can be applied to describe the energy required to increase shell area, such as those proposed in Helfrich (1973), Skalak et al. (1973), Tu and Ou-Yang (2004), and Tu and Ou-Yang (2008). However, Eq. (4.4) can be used to make easy analytical computations, and it has been shown to well represent cell behaviour at least in a certain range of deformations (Evans and Waugh 1976). For the sake of completeness, in “Appendix 2”, we report the case in which the bending energy of the membrane is considered. However, when the area of the membrane is increasing, the response is mostly dominated by the stretching of the nuclear envelope rather than by its bending (Vaziri and Mofrad 2007).

### 4.3 Solid nucleus model

To compute the energy required to deform the nucleus of the cell treated as a simple solid, we have to assume a proper constitutive equation, representing the response of the material to deformations, and calculate the deformation gradient, \( \bar{F} \).

Nowadays, a representation of the Cauchy stress tensor of cellular components is still under investigation. For the sake of simplicity, we assume an isotropic, incompressible neo-Hookean constitutive law for the nucleus of the cell, as done for instance in Caille et al. (2002). Therefore, the elastic stored energy per unit volume is given by

\[ W^V = \frac{\mu}{2} \left[ \text{tr}(\bar{C}) - 3 \right], \quad (4.7) \]

where \( \bar{C} = J^{-2/3} \bar{F}^T \bar{F}, \) \( J = \det(\bar{F}) \) and \( \mu \) is the shear modulus of the nucleus (Bonet and Wood 2008).

Both in the cases in which the deformed nucleus has an ellipsoidal shape and in the case in which it acquires a cigar-shaped configuration, we assume that parallel planes perpendicular to the axis of the cylinder in the undeformed configuration, \( Z = c \), are mapped into parallel planes in the final deformed geometry, \( z = c' \), with \( c, c' \in \mathbb{R} \). Using the standard notation of continuum mechanics, capital letters refer to quantities in the undeformed configuration, whereas lower case letters refer to quantities in the deformed configuration. Therefore, with \((X, Y, Z)\), we indicate the cartesian coordinates in the undeformed configuration and with \((x, y, z)\) the corresponding cartesian spatial coordinates. Sometimes cylindrical coordinates are used, denoted with \((\rho, \Phi, Z)\) and with \((r, \phi, z)\) in the undeformed and deformed configuration, respectively. The calculations shown in the following are based on merely geometrical considerations. All lengths are normalized with respect to \( R_n \).

#### 4.3.1 Ellipsoidal deformed nucleus

The deformation of a sphere into a prolate ellipsoid with the same volume is associated with the following deformation gradient, which is expressed in matrix form and normalized coordinates

\[ \bar{F} = \text{diag} \left\{ \frac{R_p}{R_n}, \frac{R_p}{R_n}, \frac{R^2_n}{R_p} \right\} = \text{diag} \left\{ \tilde{R}_p, \tilde{R}_p, \frac{1}{\tilde{R}_p} \right\}. \quad (4.8) \]

For this particular \( \bar{F} \), we can rewrite Eq. (4.7) as

\[ W^V = \frac{\mu}{2} \left( 2 \tilde{R}_p^2 + \frac{1}{\tilde{R}_p^4} - 3 \right), \quad (4.9) \]

which integrated over the total volume of the initial sphere, gives the total energy required to pass from the initial to the final configuration, i.e.,

\[ W^V_{\text{tot}} = \int_{V_{r}} W^V dV = \frac{2}{3} \mu \pi R_n^3 \left( 2 \tilde{R}_p^2 + \frac{1}{\tilde{R}_p^4} - 3 \right). \quad (4.10) \]

where \( V_r \) is the volume of the nucleus in the undeformed configuration taken as reference.

Equation (4.10) links the elastic energy of deformation to the mechanical properties of the nucleus, \( \mu \), the morphological properties of the nucleus \( R_n \) and the radius of the channel, \( R_p \).
4.3.2 Cigar-shaped nucleus

Experimental evidences (Friedl et al. 2011) show that sometimes the spherical nucleus deforms into a cigar-shaped nucleus, composed of a cylinder of radius \( \tilde{R}_p \) and height \( \tilde{h} = \frac{h}{R_n} \) and two hemispherical caps (see Fig. 3c).

In order to obtain the deformation gradient, we subdivide the initial spherical nucleus into three regions: The central one, of scaled height \( \tilde{H} = \frac{H}{R_n} \), is mapped into the cylindrical portion of dimensionless radius \( \tilde{R}_p \) and scaled height \( \tilde{h} \) defined by (4.3), whereas the upper and lower poles of the nucleus are mapped into the apical and basal hemispheres of the cigar-shaped nucleus.

Therefore, the deformation gradient can be described as

\[
\mathbb{F} = \begin{cases} 
\mathbb{F}_{\text{N-pole}} & \text{for } \tilde{H} \leq Z \leq 1; \\
\mathbb{F}_c & \text{for } -\tilde{H} < Z < \tilde{H}; \\
\mathbb{F}_{\text{S-pole}} & \text{for } -1 \leq Z \leq -\tilde{H}.
\end{cases}
\]

(4.11)

Assuming symmetry, we can restrict our analysis to the upper half of the nucleus, i.e., \( 0 \leq Z \leq 1 \).

To derive the deformation gradient of the central region, \( \mathbb{F}_c \), we consider a reference slice of height \( \varepsilon \) and volume \( V_r(\varepsilon) \), which is mapped into the final volume \( V_f(\varepsilon) \). Assuming that the volume is preserved and passing to the infinitesimal limit, we obtain

\[
1 = \lim_{\varepsilon \to 0} \frac{V_f(\varepsilon)}{V_r(\varepsilon)} = \lim_{\varepsilon \to 0} \frac{\pi \tilde{R}_p^2 (z(\varepsilon) + \varepsilon) - z(\varepsilon))}{\pi (1 - Z^2) \varepsilon - \pi (Z^2 \varepsilon^2 + \frac{\varepsilon^3}{3})} = \frac{\tilde{R}_p^2}{1 - Z^2} \frac{\partial z}{\partial Z}.
\]

(4.12)

which leads to

\[
\frac{\partial z}{\partial Z} = \frac{1 - Z^2}{\tilde{R}_p^2}.
\]

(4.13)

We assume that all the slices of the reference “barrel” remain parallel while deforming, i.e., \( \frac{\partial z}{\partial Z} \) is constant for all the points belonging to the same plane parallel to the \( XY \)-plane. We then consider an internal volume of the reference spherical region of height \( \varepsilon \) and volume \( V_r(\varepsilon) = \pi \rho^2 \varepsilon + o(\varepsilon) \) (for \( \varepsilon \to 0 \)), which is deformed into a volume \( V_f = \pi \rho^2 (z(Z + \varepsilon) - z(Z)) \). Keeping in mind the relation (4.13), we obtain

\[
r = \frac{\rho}{\sqrt{1 - Z^2}} \tilde{R}_p.
\]

(4.14)

Assuming \( \phi = \Phi \), for the central volume of the sphere, one has the following matrix representation of the deformation gradient in normalized bases of cylindrical coordinates (for both configurations)

\[
\mathbb{F}_c = \begin{bmatrix} \tilde{R}_p & 0 & \tilde{R}_p Z \rho \\ \sqrt{1 - Z^2} & 0 & \frac{1 - Z^2}{\tilde{R}_p^2} \end{bmatrix}.
\]

(4.15)

To fulfill the problem of describing the total deformation gradient, we have to consider the upper and lower portion of the sphere, which are mapped into the two hemispheres of the cigar-shaped nucleus. Considering a slice in these regions, in analogy with the central region, we have the following \( z \)-component of the deformation gradient

\[
\frac{\partial z}{\partial Z} = \frac{1 - Z^2}{\tilde{R}_p^2 - (z - \tilde{h})^2}.
\]

(4.16)

We remark that (4.16) holds for \( \tilde{H} \leq Z \leq 1 \) and \( \tilde{h} \leq z \leq \tilde{h} + \tilde{R}_p \).

Also, in this case, assuming that undeformed parallel planes remain parallel in the deformed configuration, we obtain

\[
r = \frac{\sqrt{\tilde{R}_p^2 - (z - \tilde{h})^2}}{\sqrt{1 - Z^2}} \rho,
\]

(4.17)

that, coupled with the hypothesis \( \phi = \Phi \), gives the following deformation gradient in cylindrical coordinates, for the upper pole of the sphere

\[
\mathbb{F}_{\text{N-pole}} = \begin{bmatrix} \frac{\sqrt{\tilde{R}_p^2 - (z - \tilde{h})^2}}{\sqrt{1 - Z^2}} & 0 & \Gamma(Z) \rho \\ 0 & \frac{\sqrt{\tilde{R}_p^2 - (z - \tilde{h})^2}}{\sqrt{1 - Z^2}} & 0 \\ 0 & 0 & \frac{1 - Z^2}{\tilde{R}_p^2 - (z - \tilde{h})^2} \end{bmatrix}
\]

(4.18)

where

\[
\Gamma(Z) = \left( \frac{Z \sqrt{\tilde{R}_p^2 - (z - \tilde{h})^2}}{(1 - Z^2)^{3/2}} - \frac{(z - \tilde{h}) \sqrt{1 - Z^2}}{\left( \tilde{R}_p^2 - (z - \tilde{h})^2 \right)^{3/2}} \right).
\]

We explicitly observe that the forms determined for \( \mathbb{F}_c \) and \( \mathbb{F}_{\text{N-pole}} \) yield a continuous \( \mathbb{F} \) across the interface separating the cylindrical region from the spherical one.

In order to express all the quantities in the material frame, we integrate Eq. (4.16), which gives the implicit relation between the Eulerian coordinate \( z \) and the corresponding material one, \( Z \)

\[
(z - \tilde{h})^3 - 3 \tilde{R}_p^2 (z - \tilde{h}) + 3(Z - \tilde{H}) - (Z^3 - \tilde{H}^3) = 0.
\]

(4.19)
Equation (4.19) is a cubic equation in \((z - \bar{h})\). In order for a solution to Eq. (4.19) to be acceptable, it has to be real and physically meaningful. It can be proven that the only physically admissible solution is determined in the case in which the determinant associated with Eq. (4.19) is negative. In this case, the solution reads

\[
z(Z) = \bar{h} + 2\bar{R}_p \times 
\cos \left[ \frac{1}{3} \cos \left( \frac{(Z - \bar{h})(Z^2 + \bar{H}^2 + Z\bar{H} - 3)}{2\bar{R}_p^3} \right) + \frac{4}{3}\pi \right].
\] (4.20)

This result can be substituted in (4.18) to get the deformation gradient in terms of the Lagrangian coordinates.

Equation (4.19) can also be used to derive \(\bar{H}\), given \(\bar{h}\). Indeed, substituting \(z = \bar{h} + \bar{R}_p\), with \(\bar{h}\) given by Eq. (4.3), and \(Z = 1\) in (4.19) we have a cubic function of the new unknown \(\bar{H}\), which gives as the only acceptable solution

\[
\bar{H} = 2 \cos \left[ \frac{1}{3} \cos \left( \bar{R}_p^3 - 1 \right) + \frac{4}{3}\pi \right].
\] (4.21)

As done for the ellipsoidal deformation, once that the deformation gradient is known, it is possible to compute the total energy required to pass from the initial configuration to the final configuration. Still assuming the constitutive law (4.7) for the nucleus of the cell, the elastic energy stored per unit volume in the central portion of the sphere is

\[
W^V = \frac{\mu}{2} \left[ 2 \frac{\bar{R}_p^2}{1 - Z^2} + \frac{\bar{R}_p^2 Z^2 \rho^2}{1 - Z^2} + (1 - Z^2)^2 \right],
\] (4.22)

whereas the energy for the upper and lower poles of the spheres is

\[
W^V_{N-pole} = W^V_{S-pole} = \frac{\mu}{2} \left[ 2 \frac{\bar{R}_p^2}{1 - Z^2} + \frac{(z - \bar{h})^2}{\left( \frac{Z}{\bar{R}_p^2} - (z - \bar{h}) \right)^{3/2}} \right]
+ \frac{\mu}{2} \left[ \frac{1}{3} \cos \left( \frac{(Z - \bar{h})(Z^2 + \bar{H}^2 + Z\bar{H} - 3)}{2\bar{R}_p^3} \right) + \frac{4}{3}\pi \right] + \frac{\mu}{2} \left[ \frac{(1 - Z^2)^2}{\left( \frac{Z}{\bar{R}_p^2} - (z - \bar{h}) \right)^{3/2}} + 3 \right].
\] (4.23)

where \(z = z(Z)\) is given by (4.20). To obtain the total energy required to pass from the initial spherical configuration to the totally deformed cell inside the channel, we have to integrate over the corresponding domains in which the deformation is experienced, i.e.,

\[
W^V_{tot} = \int_{V^e_r} W^V_{S-pole} dV + \int_{V^e_r} W^V_{N-pole} dV + \int_{V^e_h} W^V_{S-pole} dV + \int_{V^e_h} W^V_{N-pole} dV = 2 \left( \int_{V^e_r} W^V_{S-pole} hV + \int_{V^e_r} W^V_{N-pole} dV \right)
\] (4.24)

where \(V^e_r\) is the volume of the central zone in the reference configuration, \(V^N-pole\) and \(V^S-pole\) are the volumes of the north and south pole of the sphere, respectively, and \(V^e_r\) is the volume of the upper half central part of the sphere, i.e.,

\[
V^e_r = \begin{cases} 
\{ (\rho, \Theta, Z) \in \mathbb{R}^3 : 0 \leq \rho \leq \sqrt{1 - Z^2}, \\
0 < \Theta \leq 2\pi, 0 \leq Z < \bar{H} \}
\end{cases}
\]

whereas

\[
V^N-pole = \begin{cases} 
\{ (\rho, \Theta, Z) \in \mathbb{R}^3 : 0 \leq \rho \leq \sqrt{1 - Z^2}, \\
0 < \Theta \leq 2\pi, \bar{H} \leq Z \leq 1 \}
\end{cases}
\]

The previous integral can be easily computed in the central region

\[
W^V_{tot} = \mu \pi R_n^3 \left[ 2 \bar{R}_p^2 \bar{H} + \frac{1}{2} \bar{R}_p^2 \left( \frac{\bar{H}}{1 - \bar{H}^2} \right) \left( \frac{\bar{H}}{1 - \frac{\bar{H}^2}{3}} \right) \right]
\] (4.25)

We observe that since \(\bar{H}\) is a function of \(\bar{R}_p\) through (4.21), \(W^V_{tot} / \mu R_n^3\) is a function of \(\bar{R}_p\) only.

On the other hand, we can express the volume integral \(\int_{V^N-pole} W^V_{N-pole} dV\) as

\[
W^V_{tot} = \frac{\pi \mu}{2} R_n^3 \left[ 2 \int_{\bar{H}}^{1} \left( \frac{\bar{R}_p^2}{1 - Z^2} \right)^{3/2} dZ \right] + \frac{\pi \mu}{2} R_n^3 \left[ 1/2 \int_{\bar{H}}^{1} \left( \frac{Z\sqrt{\bar{R}_p^2 - (z - \bar{h})^2}}{1 - Z^2} \right)^{3/2} dZ \right]
\] (4.24)

where \(\bar{H}\) is a function of \(\bar{R}_p\) through (4.21), \(W^V_{tot} / \mu R_n^3\) is a function of \(\bar{R}_p\) only.

On the other hand, we can express the volume integral \(\int_{V^N-pole} W^V_{N-pole} dV\) as

\[
W^V_{tot} = \frac{\pi \mu}{2} R_n^3 \left[ 2 \int_{\bar{H}}^{1} \left( \frac{\bar{R}_p^2}{1 - Z^2} \right)^{3/2} dZ \right] + \frac{\pi \mu}{2} R_n^3 \left[ 1/2 \int_{\bar{H}}^{1} \left( \frac{Z\sqrt{\bar{R}_p^2 - (z - \bar{h})^2}}{(1 - Z^2)^{3/2}} \right)^{3/2} dZ \right]
\] (4.25)

\[
+ \frac{(z - \bar{h})\sqrt{1 - Z^2}}{(\bar{R}_p^2 - (z - \bar{h})^2)^{3/2}} \left( 1 - Z^2 \right)^2 dZ + \frac{(z - \bar{h})\sqrt{1 - Z^2}}{(\bar{R}_p^2 - (z - \bar{h})^2)^{3/2}} \left( 1 - Z^2 \right)^2 dZ
\]
bounded) and the geometry chosen for the deformed nucleus one. Depending on the hypothesis used to describe active

\[ \tilde{W}_{\text{active}} \geq W_{\text{tot}}^S, \]

where \( W_{\text{active}} \) is given by (4.1) and \( W_{\text{tot}}^S \) has the form presented in Eq. (4.4). Similarly, when the elastic solid nucleus model (Sect. 4.3) is applied, the nucleus can enter the cylindrical structure if

\[ W_{\text{active}} \geq W_{\text{tot}}^V, \]

where \( W_{\text{active}}^V \) has the form presented either in Eq. (4.10) for the ellipsoidal deformation or in Eq. (4.24) for the cigar-shaped one. Depending on the hypothesis used to describe active forces generated after bond formation (linear vs. constant vs. bounded) and the geometry chosen for the deformed nucleus (ellipsoid vs. cigar-shaped), inequalities (5.1) and (5.2) lead to the results presented in Table 1, with respect to the diameter ratio \( \tilde{R}_p \). \( \tilde{L}_b^{(c)} \) stands either for \( L_b = R_n \) in the case of the constant force assumption or for \( L_b^* = L_b^*/R_n \) in the bounded adhesive region case. By scaling all distances with \( R_n \) and writing all material parameters on the right-hand-side, we identify four dimensionless numbers that represent the ratios between cell active properties and nuclear mechanical parameters. In particular, for the elastic membrane model, we name

\[ G_k^* = \frac{\rho_0 \alpha_{\text{ECM}} k_b}{\lambda}, \quad G_F^* = \frac{\rho_0 \alpha_{\text{ECM}} F_M / R_n}{\mu}, \quad G_k^F = \frac{\rho_0 \alpha_{\text{ECM}} k_b R_n}{\mu}, \quad G_F^F = \frac{\rho_0 \alpha_{\text{ECM}} F_M^M}{\mu}, \]

whereas, when the elastic nucleus model is used, we introduce

\[ G_k^* = \frac{\rho_0 \alpha_{\text{ECM}} k_b R_n}{\lambda}, \quad G_F^* = \frac{\rho_0 \alpha_{\text{ECM}} F_M^M}{\mu}. \]

At the numerator, we have all the parameters that characterize active forces (densities of bonds, surface ratio of ECM, “virtual” elasticity of the cytoskeleton or maximum force that is generated after cell adhesion to the ECM), whereas at the denominator, we have the parameter describing the mechanical properties of the cell nucleus (\( \lambda \), in the case of an elastic membrane, \( \mu \), in the case of an elastic solid).

In Table 1, we name

\[ I(\tilde{R}_p) = \frac{W_{\text{tot}}^V}{3 \pi \mu R_n^3} = \frac{W_{\text{tot}}^{\text{N-Pole}}}{4 \pi \mu R_n^3} + 2 \frac{W_{\text{tot}}^{\text{N-Pole}}}{3 \pi \mu R_n^3}. \]

The right-hand side of each relation identifies the critical value of the characteristic number, and it is indicated in the following with \( G_i^j \) (with \( i = \{ \lambda, \mu \}, j = \{ k, F \} \)). Therefore, once a proper model is chosen, for every diameter ratio \( \tilde{R}_p \), it is possible to define the value of \( G_i^j \), above which a cell, with a nucleus of dimension \( R_n \) can enter a channel of radius \( \tilde{R}_p R_n \).

### Table 1 Energy-based criteria

| Model                        | Linear force | Constant (bounded) Force |
|------------------------------|--------------|--------------------------|
| Elastic membrane and liquid droplet |              |                          |
| Ellipsoid                    | \[ G_k^* \geq \frac{16\pi}{3} \frac{\tilde{R}_p^2 \left( 1 + \frac{\alpha(\epsilon)}{\tilde{R}_p^3} \right) - 1}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{ellips}}^c} \] | \[ G_F^* \geq \frac{8\pi}{3} \frac{\tilde{R}_p^2 \left( 1 + \frac{\alpha(\epsilon)}{\tilde{R}_p^3} \right) - 1}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{ellips}}^c} \] |
| Cigar                        | \[ G_k^* \geq \frac{16\pi}{3} \frac{\left( \frac{1}{3} \tilde{R}_p^2 + \frac{2}{3} \tilde{R}_p - 1 \right)^2}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{cigar}}^c} \] | \[ G_F^* \geq \frac{8\pi}{3} \frac{\left( \frac{1}{3} \tilde{R}_p^2 + \frac{2}{3} \tilde{R}_p - 1 \right)^2}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{cigar}}^c} \] |
| Elastic solid nucleus        |              |                          |
| Ellipsoid                    | \[ G_k^* \geq \frac{2}{3} \frac{\tilde{R}_p^2 + 1}{\tilde{R}_p^3} - \frac{3}{3} \] | \[ G_F^* \geq \frac{2}{3} \frac{\tilde{R}_p^2 + 1}{\tilde{R}_p^3} - \frac{3}{3} \] |
| Cigar                        | \[ G_k^* \geq \frac{4}{3} \frac{I(\tilde{R}_p)}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{cigar}}^c} \] | \[ G_F^* \geq \frac{2}{3} \frac{I(\tilde{R}_p)}{\tilde{R}_p \tilde{L}_b^{(c)} \Delta L_{\text{cigar}}^c} \] |

The dimensionless numbers \( G_i^j \) represent the ratio between cell active properties and nuclear mechanical parameters. In particular, \( G_k^* = \frac{\rho_0 \alpha_{\text{ECM}} k_b}{\lambda}, \ G_F^* = \frac{\rho_0 \alpha_{\text{ECM}} F_M / R_n}{\mu}, \ G_k^F = \frac{\rho_0 \alpha_{\text{ECM}} k_b R_n}{\mu}, \ G_F^F = \frac{\rho_0 \alpha_{\text{ECM}} F_M^M}{\mu} \).
Fig. 5 Elastic membrane model: a $\overline{G}_\mu^k$ in the case of linear forces, b $\overline{G}_\mu^F$ in the case of constant forces and c $\overline{G}_\mu^F$ in the case of constant forces over a bounded region, with $L_b^M = 5$. The curves indicate the minimum value of the characteristic numbers that need to be overcome in order for the cell to enter a channel of radius $\tilde{R}_p$

We remark that this model, taking into account the finiteness of the nuclear dimensions, is valid only for $\tilde{R}_p \leq 1$. Indeed, when $\tilde{R}_p \rightarrow 1$, the elastic energy required to deform the nucleus vanishes and the cell is easily pulled inside the channel, without nucleus deformation.

Figure 5 shows the value of (a) $\overline{G}_\lambda^F$ in the case of linear forces, (b) $\overline{G}_\lambda^F$ in the case of constant forces and (c) $\overline{G}_\lambda^F$ for constant force over a bounded adhesive region, above which the cell can enter a channel of scaled radius $\tilde{R}_p$, when the elastic membrane model is used. On the other hand, Fig. 6 reports the ratios (a) $\overline{G}_\lambda^k$ and (b–c) $\overline{G}_\lambda^F$ obtained applying the elastic solid nucleus model, under the same hypothesis of active forces.

Both in Figs. 5 and 6 solid lines represent ellipsoidal deformations, whereas dashed lines stand for cigar-shaped final configurations. In any case, the assumption on the geometry acquired by the deformed nucleus does not affect the qualitative behaviour of the solutions.

We remark that in Fig. 5a, b, for very small radius, the energy required to increase the area of the nuclear membrane increases, but the work done by active forces increases faster and therefore, for $\tilde{R}_p \rightarrow 0$, $\overline{G}_\lambda^k$ and $\overline{G}_\lambda^F$ go to zero when the unbounded $L_b$ is used, giving rise to the contradiction that cell can easily enter channels of very small diameters.

Indeed, when the radius of the channel is small, the length of the deformed cytoplasm grows considerably, leading, in the linear case, to active traction forces that are unrealistically high. At the same time, the area of the nuclear membrane increases considerably as the channel size decreases. Figure 7 plots the increment in the surface area over the channel radius, for the two different morphologies. We remark that in this model, no limitation is imposed on membrane extension.

In particular, for $\tilde{R}_p \rightarrow 0$, we have that the energy required to deform the elastic membrane increases as $R_p^{-2}$, given that the increase in the surface area grows as $\tilde{R}_p^{-1}$. On the other hand, at the denominator of $\overline{G}_\mu$, we have $\Delta \tilde{L} = O \left( \tilde{R}_p^{-2} \right)$ and $\tilde{L}_b = O \left( \tilde{R}_p^{-2} \right)$. Therefore, when the linear force assumption is used, $\overline{G}_\lambda^k = O \left( \tilde{R}_p^3 \right)$ for $\tilde{R}_p \rightarrow 0$, whereas, when the bond force is assumed constant, $\overline{G}_\lambda^F = O \left( \tilde{R}_p \right)$ for $\tilde{R}_p \rightarrow 0$.

In order to avoid unphysical results, we more realistically assume that bonds are formed on the surface of the channel until the maximum length, $L_b^M$ is reached. Restricting the extent for which bonds are formed to a certain length ($L_b = L_b^*$) will dramatically limit the work done by active forces for very small $\tilde{R}_p$. In this case, for $\tilde{R}_p \rightarrow 0$, $L_b = O \left( 1 \right)$ and thus the critical $G_{\lambda}^F$ goes to infinity like $\tilde{R}_p^{-1}$ (see Fig. 5c).

Unrealistic results are obtained also using the linear force model coupled with the elastic solid nucleus model (see Fig. 6a). In this case, for very small radii, the energy required to deform the elastic nucleus is $O \left( \tilde{R}_p^{-4} \right)$, and hence, under the linear force assumption, the critical $G_{\mu}^k$ goes linearly for $\tilde{R}_p \rightarrow 0$. On the other hand, when a constant force assumption is used, we have that $\overline{G}_\mu^F$ goes to infinity as $\tilde{R}_p^{-1}$, whereas limiting the adhesive region, it goes like $\tilde{R}_p^{-3}$, for $\tilde{R}_p \rightarrow 0$.

Figure 6b reports the results obtained applying the elastic solid nucleus model with the constant force assumption, whereas Fig. 6c is obtained under the boundedness assump-
Fig. 6 Elastic solid nucleus model: a $\overline{G}_\mu^F$ in the case of linear forces, b $\overline{G}_\mu^F$ in the case of constant forces and c $\overline{G}_\mu^F$ in the case of constant forces over a bounded region (with $\bar{L}_b^M = 5$). The curves indicate the minimum value of the characteristic numbers that need to be overcome in order to have the cell enter a channel of radius $R_p$.

Fig. 7 Dimensionless surface area increase, $\Delta \bar{S} = \frac{\Delta S}{4\pi R_n^2}$ of a nucleus entering inside a channel of dimensionless radius $\bar{R}_p$. The calculation is done both for the ellipsoidal deformed nucleus ($\Delta S = \Delta S_{\text{ellips}}$) and the cigar-shaped nucleus ($\Delta S = \Delta S_{\text{cig}}$). deformation is very small, it is possible to use the analytical relation obtained for the ellipsoidal case

$$\overline{G}_\mu^F = \frac{2}{3} \left[ \frac{2\bar{R}_p^2 + \frac{1}{\bar{R}_p^4} - 3}{\bar{R}_p L_b^{(s)} \Delta L_{\text{ellips}}} \right].$$

(5.3)

We remark that the r.h.s. of (5.3) is a decreasing function of $\bar{R}_p$; thus, the value of $\overline{G}_\mu^F$ increases with decreasing channel radii. Recalling that $G_\mu^F$ represents the ratio between cell active and nuclear mechanical properties, Eq. (5.3) states that, in order to enter a channel of given radius, cells with a stiffer nucleus (greater $\mu$) should either increase the number of adhesive bonds ($\rho_b$) or the number of focal points in contact with ECM ($\alpha_{\text{ECM}}$) or even the active force ($F_M^b$), with respect to cells with softer nuclei.

This finding is in qualitative agreement with a number of experimental works, such as (Beadle et al. 2008; Rolli et al. 2010; Wolf et al. 2007), where the cell migratory capability is associated with nuclear deformations, and the existence of a critical channel radius has been observed (here, by “critical radius”, it is meant the value of the channel radius above which a cell with certain elastic properties can enter the channel). Moreover, it is comparable with the results obtained with discrete model (Scianna et al. 2013; Scianna and Preziosi 2013), confirming that mechanical properties of the nucleus can affect the cell entry into channels.

Equation (5.3) can be of great value, for instance, in scaffold design. Indeed, assuming that cell mechanical properties and their capabilities to express bonds are known, it is possible to evaluate the pore size that allows the cells to penetrate the rigid network.
6 Discussion and conclusions

Due to the increasingly recognized importance of understanding cell migration processes in 3D extracellular environments and to its exploitations, e.g., in tissue engineering, theoretical models are needed to be able to analyse the relative influence of single and interrelated parameters on the overall migratory process.

We identified some energy-based criteria that take into account the mechanical properties of cell nucleus, the adhesive characteristics of the cell membrane, the active force generated through cytoskeleton contraction, the finiteness of the nucleus and the aspect ratio of the structures involved in cell migration. In doing this, we tried to maintain the model as simple as possible in order to obtain easily manageable results.

For the examples presented, some analytical results are obtained, providing the relation between active and mechanical properties that should be satisfied in order to have cells entering a channel of given radius. Therefore, knowing the adhesive, mechanical and contractile properties of the cell, it is possible to derive the minimum channel size and, conversely, observing experimentally the capability of a cell to enter cylindrical channels of different dimensions, it is possible to characterize the interplay between mechanical and active properties.

Our results predict that cells are able to enter ECM-networks only for pore radii bigger than a critical value, depending on the stiffness of their nucleus and their capabilities of expressing adhesion molecules in order to bind to the extracellular matrix. Indeed, a rigid cell body would nullify any attempt of the cell to squeeze through channels and network gaps narrower than the nucleus dimension, as observed in Rolli et al. (2010), Wolf et al. (2007).

In order to obtain reliable quantitative results, more studies are required, from both the biological and the mathematical point of view. In particular, more experiments are needed in order to characterize the mechanical behaviour of cells and a proper relation for the active force exerted through cytoskeleton contraction. In this respect, the application of the method developed in Vitale et al. (2012) can help evaluating the localization and the magnitude of the traction forces exerted by a cell moving in a 3D network of fibres. A more comprehensive understanding of the microscopical mechanisms regulating nucleus deformation and cytoskeletal reorganization, when the cell is anchored to ECM, can also help to obtain a more realistic description of the overall deformation process.

Moreover, biological experiments would be very useful to validate the model presented in this paper. First of all, a mechanical characterization of cell nuclei should be accomplished (see for instance Caille et al. 2002) in order to assess the range of validity of the Neo-Hookean law used here to represent the nuclear constituent and, if needed, to determine even more suitable constitutive equations. Should more sophisticated constitutive laws be necessary, the energetic approach presented in our paper could still be used by simply following the procedure outlined up to this point, since the functional form of the deformation gradients is independent on the chosen constitutive law. Of course, more complex constitutive laws will involve a larger number of parameters and could, thus, make a comparison with biological data more burdensome. Indeed, also in those works in which other constitutive equations for the nucleus are postulated, such as the Mooney–Rivlin law, then some simplifications are made in order to get back the neo-Hookean law, characterized by a unique parameter (Caille et al. 2002).

To our knowledge, the most critical point in the validation process, both from the biological and the mathematical point of view, is the determination of the forces acting on the nucleus. Nowadays, many experimental settings have been designed in order to measure traction forces on the surrounding ECM (Wang and Lin 2007), but the force transmitted by cytoskeletal filaments to the nucleus is much more difficult to be assessed, due to the highly complex intracellular structure.

The determination of the active forces that make the nucleus deform is also a critical issue from the mathematical point of view. The mathematical translation of biological information, even when data are available, might not be straightforward, due to the high complexity and the high heterogeneity of the involved biological structures.

Indeed, in this work, we do not go into details in the complex process of cytoskeleton contraction and active force generation. We here focus only on some possible forms of the total force exerted on the nucleus, after the formation of bonds between the cell and the ECM. These relations are kept as simple as possible in order to obtain analytical results and to avoid increasing the number of model parameters too much. Thus, a more detailed description of the intracellular dynamics of cytoskeletal contraction and polymerization should be introduced in the model, as done for example in Hawkins et al. (2009) (where the nucleus mechanics is not described) for adhesion-independent movements. However, introducing more complicated models for traction forces and considering other mechanisms of motion will increase the number of parameters involved.

Moreover, cells are known to implement different mechanisms of motion, which can be all present (Lämmermann et al. 2008; Renkawitz and Sixt 2010). Indeed, we have to be aware that the transmigration of cells in 3D environments does not always require the cell to stick to the substrate (Lämmermann et al. 2008; Renkawitz and Sixt 2010), even though integrin-mediated motion is necessary while moving in 2D environments. Future works should also focus on the cell capability of switching between adhesion-receptor-mediated force transmission (as the one considered here) and mechanisms of locomotion based on cellular deformation and...
Fig. 8 Possible applications of the results presented in this paper: (on the left) design of scaffolds and microchannels, with optimal pore size and (on the right) identification of the null flow region depending on cell properties and scaffold porosity. Figure 8-left represents, for a given value of $G^F_\mu$ (light blue solid line), the minimum value of the channel radius that allows the nucleus to enter, $\tilde{R}^c_p$. In Fig. 8-right, the dotted region represents the set of all pairs $(\tilde{R}^c_p, G^F_\mu)$ for which no microscopic (and thus macroscopic) cellular motion can occur, because $G^F_\mu$ is below the critical value (red curve).

bleb formation, which are independent of adhesion receptors (Renkawitz and Sixt 2010). Furthermore, in our ongoing work, we would like to study the whole dynamic process, considering all the steps of the cell entering the channel. Indeed, the model we proposed here is based on an “integral” approach, i.e., it considers the total work required to bring the nucleus from the initial undeformed configuration to the final deformed one and, thus, it gives an estimate of the “mean” active force, scaled by the mechanical deformability of the nucleus, required to accomplish this task. However, this method does not take into account the possible existence of intermediate stages in which the force needed to squeeze the nucleus inside the channel could be larger than the maximum active force that can be possibly exerted. That is, the integral criterion, used here to obtain analytical expressions, gives a necessary but not sufficient condition for the passage of the cell inside the channel. Therefore, a criterion able to establish the maximum force needed would be more precise, though it requires 3D time-dependent numerical simulations. With the aid of finite element simulations, it would be possible to consider the deformation of both the nucleus (as done in Vaziri and Mofrad (2007) for instance) and the cytoplasmatic region. Furthermore, it would be interesting to include the deformability of the surrounding extracellular structure, which here has been supposed rigid.

Furthermore, we should be aware that for very small channel size, the hypotheses used to derive the model lose their validity, both in the case of the elastic membrane and in the case of the elastic solid nucleus. Indeed, for what concerns the elastic membrane model, no constraints are imposed on the maximum admissible extensibility above which the nucleus membrane breaks and thus the membrane area is let free to expand without constraints (see Fig. 7). Even in the elastic solid model, it is known that mechanical properties of nuclear constituents show plastic behaviour when their elongation is above a threshold (see Houchmandzadeh et al. 1997). Moreover, recent studies (Friedl et al. 2011; Rowat et al. 2006; Versaevel et al. 2012) have shown that the volume of the nucleus is no longer preserved when large elongations occur, suggesting that the nuclear envelope is permeable to aqueous material. In particular, in Rowat et al. (2006), it was shown that during the total aspiration of a cell inside a pipette, chromatin and other macromolecules remained in the nucleus, while water and smaller solutes (e.g., salts) were exchanged with the cytoplasm, through the nuclear membrane, generating volume loss for the nucleus.

Therefore, future works should focus on a more detailed description of the nucleus and cell behaviours under intense deformations, considering the existence of a limit surface area above which the nuclear membrane can no more extend as well as the decrease in the nuclear volume, followed by changes in the mechanical properties.

In spite of all possible developments, the energetic framework presented here is quite general and continues to be valid even for more complex cell and membrane constitutive assumptions, providing some interesting preliminary results. From the mathematical point of view, the results presented can help in the description of an ensemble of cells in the surrounding environment. Nowadays, in order to describe from the macroscopic point of view the movement of a population of cells inside a region containing extracellular matrix, Darcy’s Law is generally used (cf., for example, Chauvière et al. 2007a, b; Lowengrub et al. 2010; Painter 2009; Preziosi and Vitale 2011; Verdier et al. 2009), so that the velocity of the ensemble of cells depends on a permeability coefficient $k$ that is generally a function of the volumetric fraction of
the ECM. The results presented in our paper show that ascertaining the validity of Darcy’s law requires to determine the influence of the microscopic mechanical properties of single cells on the overall cellular motion in addition to the knowledge of the geometry and dimension of the pores. This statement becomes clear if one looks at Fig. 8-right, where the dotted region represents the set of all pairs \((\tilde{R}_p, G^F_{\mu})\) for which no microscopic (and thus macroscopic) cellular motion can occur. In addition to that, Fig. 8-left represents, for a given value of \(G^F_{\mu}\), the set of radii \(\tilde{R}_p \in [\tilde{R}_p^1, 1]\) for which \(\bar{G}^F_{\mu}(\tilde{R}_p) \leq G^F_{\mu}\) and, consequently, a cell can enter a channel. Therefore, from the biomechanical point of view, the approach described in our paper could be applied to the design of synthetic scaffolds, with optimal values of pore size and fibre density, that may accelerate cell transport and ingrowth, critical for regenerative treatments (see Fig. 8-right).

Appendix 1: Micropipette models applied to cell migration inside channels

For sake of completeness, we consider here the case in which nucleus entry obeys the classical relations (2.2) or (2.5), deforming the initial spherical nucleus into a cigar-like shape, with the assumption that \(L_p\) in (2.2) and (2.5) represents the length of the deformed nucleus, i.e., \(L_p = L^\text{fin}_{n,cigar} = 2(h + R_p)\) with \(h\) given by Eq. (4.3). We define the critical pressure as the value of \(\Delta P\) for which \(L_p = L^\text{fin}_{n,cigar}\) and we assume that a proper representation for \(\Delta P\) in Eqs. (2.2) and (2.5) is \(F^Z_{\text{active}}/\pi R_p^2\), where \(F^Z_{\text{active}}\) is the \(Z\)-component of the active force given either by Eq. (3.8) or (3.9) or (3.10). Then, assuming that a pressure above the critical one makes the cell move inside the pipette, it is possible to obtain the relation between mechanical and active properties that should hold for the cell to enter the channel, depending on the geometrical properties (i.e., \(R_n\), \(R_c\) and \(R_p\)).

The inequalities that should be satisfied in each case are summarized in Table 2, as a function of the diameter ratio \(\tilde{R}_p = R_p/R_n\). On the left-hand side of each relation, we have characteristic parameters representing the ratio between active properties and mechanical properties of cell nucleus. In particular, we identify

\[
G^{k}_\gamma = \frac{\rho_b \alpha_{\text{ECM}} k_b R_n^2}{\gamma}, \quad G^{F}_\gamma = \frac{\rho_b \alpha_{\text{ECM}} F^M_{b} R_n}{\gamma},
\]

\[
G^{k}_E = \frac{\rho_b \alpha_{\text{ECM}} k_b R_n}{E}, \quad G^{F}_E = \frac{\rho_b \alpha_{\text{ECM}} F^M_{b} R_n}{E}.
\]

On the right-hand side of each relation, we have the critical value of the characteristic number (indicated with \(\bar{G}^{F_i}\), with \(i = \{\gamma, E\}, j = \{k, F\}\)), which is a function of the diameter ratio, and we have set

\[
\tilde{L}_p = \frac{L_p}{R_n} = \frac{2}{3} \tilde{R}_p \left[ 1 + 2 \left( \frac{1}{R_p} \right)^3 \right],
\]

\[
\tilde{L}_b = \frac{L_b}{R_n} = \tilde{R}_p \left[ \frac{4}{3} \left( \frac{R_c}{R_p} \right)^3 - \frac{1}{R_p^3} \right] + \frac{1}{3} + \frac{(L^0_n)^2}{R_p^3} \left( 1 - \frac{1}{3} L^0_n \right)
\]

with \(L^0_n = 1 - \sqrt{1 - R_b^2}\) and \(\tilde{R}_c = R_c/R_n\).

The critical characteristic numbers are plotted in Fig. 9 as a function of the diameter ratio of the channel. The graphs represent the minimum value that each constant should assume in order to have the cell totally inside the channel, according to Chien’s criterion (Fig. 9a) and Theret’s one (Fig. 9b). Results obtained with the linearized Chien’s equation (2.4) are comparable with the ones obtained with the more complex formula (2.2). In Fig. 9, the dashed line represents results obtained using constant forces over a bounded domain (where we set \(L_b^* = 5\)). It is possible to see that for big \(\tilde{R}_p\), \(\bar{G}^{F}_\gamma\) and \(\bar{G}^{F}_E\) are obviously not influenced by the assumption on the boundedness of the contact region in which integrals are expressed (i.e., the red-dashed curve and the black-solid one overlap). Indeed, it exists an \(\tilde{R}_p^*\) such that \(L_b^* = L_b\) for \(\tilde{R}_p > \tilde{R}_p^*\), whereas \(L_b^* = L_b^M\) for \(\tilde{R}_p < \tilde{R}_p^*\). Therefore, the active work is influenced by the boundedness assumption only for \(\tilde{R}_p < \tilde{R}_p^*\).

For instance, Fig. 10 explains how these graphs can be interpreted (for the particular case of Chien model): The bar charts below the graph represent the range of \(\tilde{R}_p\) for which a cell characterized by either a given \(G^{k}_\gamma\) or a given \(G^{F}_\gamma\) can enter the channel.

| Model | Linear force | Constant (bounded) force |
|-------|--------------|--------------------------|
| Chien | \(G^{k}_\gamma \geq \frac{2 \tilde{L}_p}{R_p} - 1 + \log \left( \frac{2 \tilde{L}_p}{R_p} \right)\) | \(G^{F}_\gamma \geq \frac{2 \tilde{L}_p}{R_p} - 1 + \log \left( \frac{2 \tilde{L}_p^{(e)}}{R_p} \right)\) |
| Theret | \(G^{k}_E \geq \frac{2 \pi}{3} \Phi \frac{\tilde{L}_p}{L_b}\) | \(G^{F}_E \geq \frac{2 \pi}{3} \Phi \frac{\tilde{L}_p}{L_b^{(e)}}\) |
Fig. 9 Critical value of the characteristic numbers obtained applying a Chien’s model and b Theret’s model, under either a linear force (blue) or a constant force (black) or a constant force over a bounded region (red dashed) assumption.

Fig. 10 Interpretation of the results: bar charts represent the range for which a cell, with a given $G^k_\gamma$ or $G^F_\gamma$, can enter the channel, for the different hypotheses of bond forces.

In the figure, ‘cell 1’ (orange) is characterized by higher $G^k_\gamma$ or $G^F_\gamma$ than ‘cell 2’ (violet). This means that we are considering either a softer cell (i.e., smaller $\gamma$) or a cell that is able to establish a higher number of adhesive bonds (i.e., higher $\rho_b\sigma_{ECM}$) or a cell with better contractile capabilities (i.e., bigger $k_b$ or $F^M_b$). In any case, the range for which ‘cell 1’ can enter the pipette is bigger than for ‘cell 2’ (orange bars vs. violet bars), according to what we expect from biological observations. Moreover, using the constant force assumption, it is possible to see that the range for which cells can enter the pipette is bounded both from below and from above. On the other hand, using the linear force assumption, we do not have any inferior limit, in contrast with biological observation. This contradictory result is due to the hypothesis used in the representation of forces. Indeed, in this case, the more the cytoplasm of the cell spreads inside the channel (small $R_p$), the more the traction force (which is related to the adhesive region) can pull the nucleus inside. In particular, even though the force required to deform the nucleus grows as $R_p^{-3}$, as $R_p \to 0$, the adhesive-dependent active force raises faster, since $L^2_b = O(R_p^{-4})$. On the other hand, when a constant force assumption is used, for small $R_p$, the length for which bonds are formed augments ($L^2_b = O(R_p^{-2})$ for $R_p \to 0$). Thus, the total integrin-dependent traction force exerted on the nucleus increases, but it is not sufficient to compensate the greater deformation required by the nucleus, which goes like $R_p^{-3}$ for $R_p \to 0$. Conversely, introducing the boundedness assumption on $L_b$, the active force is limited.

In particular, we have that for $R_p \to 0$, $G^F_\gamma$ goes like $R_p^{-\alpha}$ (with $\alpha = 1$ for unbounded $L_b$ and $\alpha = 3$ when the adhesive region is limited) and $G^k_\gamma$ grows linearly.

On the other hand, when the radius of the pipette is very big, the entry of the cell into the channel is limited due to the decrease in the contact area between the cell and the channel wall, where adhesive bonds are formed. It is likely that, in this case, the force exerted by actomyosin is not equal to the maximum executable force. Thus, a linear force can better describe the physiological behaviour. Therefore, a good choice for the bond force relation could be a ramp force on a bounded adhesive region, which is also the most conservative case.
In Theret’s model, it is possible to see that, for $\tilde{R}_p \to 0$, $\mathcal{U}_E^k = \mathcal{O} \left( \tilde{R}_p^2 \right)$ and $\mathcal{G}_E = \mathcal{O} \left( 1 \right)$ when the constant force assumption with unbounded adhesive region is implemented. Thus, neither the constant force assumption nor the linear force one can account for the inferior limit in pipette calibres. Only enforcing the boundedness of the adhesive region, the capability of cells to enter very small channels is prevented.

Both Chien’s and Theret’s models, with the assumption of active forces over a bounded region, provide evidence for a biphasic cell migratory behaviour that reveals most optimal migration at pore sizes at nuclear and subnuclear diameters and diminishes at gaps greatly bigger or smaller than the cell nucleus diameter.

However, even though the results obtained by applying the classical models above seem promising, especially when adhesion is active on a bounded domain, they cannot account for the finite boundaries of the nucleus. Indeed, Chien’s model refers to an infinite 2D membrane, whereas Theret’s one was derived for a 3D half-space aspired inside a pipette, only for a small portion. Therefore, these criteria cannot be applied to describe the total entry of the cell into a pipette. The consequence of this assumption is evident in Fig. 9, where, for $\tilde{R}_p = 1$, the force needed to deform the nucleus does not vanish.

**Appendix 2: Influence of bending**

In Sect. 4.2, we considered only the contribution to the surface deformation energy due to the stretching of the nuclear membrane, but we disregarded the energy contribution associated with bending. In order to introduce in the model the bending of the nuclear membrane, we refer to Helfrich’s work on lipid bilayers (Helfrich 1973). Helfrich introduced a model (Helfrich 1973) in which the bending energy of a membrane is given by

$$ W_{\text{bending}} = \frac{k_c}{2} \int (2H - c_0)^2 \, dS + \frac{k_g}{2} \int K \, dS, \quad (6.1) $$

where $H = \frac{1}{2}(k_1 + k_2)$ is the mean curvature of the membrane surface, $S$, $k_1$ and $k_2$ are the principal curvatures, $K = k_1 k_2$ is the Gaussian curvature, and $c_0$ represents the spontaneous curvature that describes the asymmetric effect of the membrane. We remark that, from the Gauss-Bonnet theorem, the second term in the Helfrich energy is a topological invariant, and thus, in this work, it can be omitted (Laadhari et al. 2010). The total energy of deformation of the nuclear membrane, considering both the contribution due to stretching and the one due to bending, is Landau and Lifschitz (1986)

$$ W_{\text{tot}}^S = \lambda (\Delta S)^2 + W_{\text{deformed}} - W_{\text{bending}}, \quad (6.2) $$

where $W_{\text{bending}}^{\text{sphere}} = 8\pi k_c$. For the cigar-shaped deformed configuration, we define

$$ W_{\text{bending}}^{\text{deformed}} = W_{\text{bending}}^{\text{cigar}} = 2\pi k_c \frac{h}{R_p} + 8\pi k_c, \quad (6.3) $$

whereas, for a nucleus deformed into an ellipsoid, we have

| Table 3: Energy-based criteria: stretching and bending |
|------------------------------------------------------|
| Deformation | Force | Criteria |
|-------------|-------|----------|
| Cigar-shaped |
| Linear force | $G_k^k \geq \frac{16\pi}{3} \left( \frac{1}{2} \tilde{R}_p^2 + \frac{2}{3R_p} - 1 \right)^2 + \tilde{k}_c \left( \frac{1}{R_p} ight)^2 + \frac{\tilde{h}}{R_p}$ | |
| Constant (bounded) force | $G_k^F \geq \frac{8\pi}{3} \left( \frac{1}{2} \tilde{R}_p^2 + \frac{2}{3R_p} - 1 \right)^2 + \tilde{k}_c \left( \frac{1}{R_p} ight)^2 + \frac{\tilde{h}}{R_p}$ | |
| Ellipsoid |
| Linear force | $G_k^k \geq \frac{16\pi}{3} \left( \frac{1}{2} \tilde{R}_p^2 + \frac{2}{3R_p} - 1 \right)^2 + \tilde{k}_c \left( \frac{1}{R_p} ight)^2 + \frac{\tilde{h}}{R_p}$ | |
| Constant (bounded) force | $G_k^F \geq \frac{8\pi}{3} \left( \frac{1}{2} \tilde{R}_p^2 + \frac{2}{3R_p} - 1 \right)^2 + \tilde{k}_c \left( \frac{1}{R_p} \right)^2 + \frac{\tilde{h}}{R_p}$ | |
Fig. 11 Critical value of $G_k^L$ and $G_k^F$ for different values of $k_c$, assuming a cigar-shaped deformation

Fig. 12 Critical value of $G_k^L$ and $G_k^F$ for different values of $k_c$, assuming an ellipsoidal deformation

\[
\gamma_{\text{deformed}} = \frac{k_c}{2} \int_{s_{\text{ellips}}} (2H)^2 dS = \frac{k_c \pi}{2} \int_0^\pi (2H)^2 A \sin \Theta, \quad (6.4)
\]

where we referred to Poelaert et al. (2011), in which the principal curvatures of the general ellipsoid are derived, i.e.,

\[
2H = \frac{F^2 (h_c^2 + 2R_p^2 - R^2)}{h_c^2 R_p^4}, \quad (6.5)
\]

\[
A = h_c^2 R_p^2 \sqrt{R_p^4 \cos^2 \Theta + h_c^4 \sin^2 \Theta} \sin \Theta, \quad (6.8)
\]
and $h_e = R_n \frac{R^2}{R_p^2}$ is defined in (4.2). The integral (6.4) is solved numerically.

Once $\gamma_{tot}^0$ is known, it is possible to obtain an expression for the dimensionless parameters $G^\lambda_\lambda$ and $G^\xi_\xi$. The results are reported in Table 3, where $k_c = \frac{k_c}{\lambda R_n^4}$, $Q(R_p) = \int_0^{\pi/2} (2H)^2 \pi d\Theta$, $G^\lambda_\lambda$ and $G^\xi_\xi$ have the same definition given in Sect. 5.

The results are reported in Fig. 11 for the cigar-shaped nucleus and in Fig. 12 for the ellipsoidal nucleus. In order to observe the bending contribution, the ratio $k_c/\lambda$ should be larger than 10μm$^4$. However, from Helfrich (1973), we, know that for lipid bilayer membrane of a nucleus of radius 4μm, we have $k_c/\lambda = 4 \times 10^{-5}$μm$^4$. Thus, under the assumption that the surface of the nucleus increases, the major contribution is due to stretching.

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