A viscoelastic nuclear model predicting mechanical memory and convergence rate of nuclear spreading as a function of stiffness

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Abstract.

Cellular processes are directly controlled by the mechanics of the extracellular matrix. External mechanical signals are directly transmitted from the adhesion complex to the nucleus via the actomyosin apparatus, stressing the organelle and causing reversible and irreversible phenomena. To fully understand the dynamics of this nuclear strain responsible for important mechanoresponsive behaviors, it is crucial to build a model that considers all the nuclear mechanical properties that have been reported to impact nuclear strain. We developed a model integrating the viscoelastic property of the nucleus caused by heterochromatin and lamins in order to consider the time-dependent contributions of such important nuclear elements. The model managed to predict the contribution of lamin-A,C and lamin-B to the nuclear strain and stress for different ECM stiffnesses as previously shown by others experimentally. The model also suggests a possible dynamic role of lamins levels as an explanation of cell mechanical memory due to the irreversibility of these modifications. And finally, the model seems to point towards a need to experimentally study the kinetics of nuclear strain to better understand ECM-stiffness-related mechanisms on cell nuclei, as the timescale during which stress is applied is of great relevance in defining the future of a cell.

Introduction.

Adherent cells are constantly sensing their three-dimensional microenvironment through several internal and external mechanisms that are then converted into an adaptive response involving possible changes

in cell mechanics, shape, internal structure, functions and even fate (Chen et al., 2012; Engler, Sen, Sweeney, & Discher, 2006; Wolfenson, Yang, & Sheetz, 2018). It has also been demonstrated that cells generate forces that seem to be responsible for their internal structural organization (Discher, Janmey, & Wang, 2005; Livne & Geiger, 2016) and their necessary non-equilibrium but stable state (quiescence) is guaranteed by the permanent dynamic reorganization of their cytoskeleton, that maintains cell shape and function (Ahmed, Fodor, & Betz, 2015). Understanding this particular and very complex loop phenomenon of sensing, transducing and reacting is critical to model the dynamic activity of cells. Many competing or symbiotic processes must be taken into account and studied, starting from the mechanical properties of the extracellular matrix (ECM), such as concentration and cross-linking, its composition, and the density and maturity of focal adhesions interacting with the ECM proteins (Holle et al., 2018), cell-to-cell interactions and cellular junctions (Hoffman & Yap, 2015), active assembly and disassembly of actin filaments and positive feedback loop of actomyosin (Livne & Geiger, 2016). All this inputs resulted in changes of the cells stiffness as a response to their environment (DuFort, Paszek, & Weaver, 2011; Solon, Levental, Sengupta, Georges, & Janmey, 2007). Other intrinsic properties of cells such as its mechanical pre-stress or tensegrity (Lee et al., 2015; Wang, Tytell, & Ingber, 2009) and chemomechanics are also considered (Graham & Burridge, 2016) to try and build better predictive models of cell behavior in mechanobiology. Today, the mechanics of the nucleus (Lammerding, 2011) and its interactions with the cytoskeleton and its internal forces are better understood and it is known that its behavior and response are of the greatest importance in the definition of the whole cell function and phenotype (Skinner & Johnson, 2017). It has also been shown that the evolution of nuclear shape and its orientation need to be included in the mechanistic description of cell spreading (Y. Li et al., 2015), migration (Raab & Discher, 2017), polarization (Shenoy, Wang, & Wang, 2016), differentiation (Smith et al., 2017) and attachment-detachment (D.-H. Kim et al., 2016). Hence, the nucleus is increasingly described as an important mechanosensing unit of the cell, with morphological properties intrinsically correlated to cell functions and behavior (Smith et al., 2017; Uhler & Shivashankar, 2017; Wang et al., 2009), e.g. a direct correlation was found between the dimensions of cells and the corresponding dimensions of their nucleus in mesenchymal stem cells (MSCs) (Buxboim et al., 2017).

For better description models to be built accurately, the interactions of the nucleus with the cytoskeleton and the impact of the shape and position of the nucleus are now extensively studied (Lele, Dickinson, & Gundersen, 2018). Recent experimental findings in this field have demonstrated the need to consider the mechanical transduction coupling between the building blocks of the cytoskeleton and the nucleus, as mechanical forces are transmitted from the outside to the center of the cells via a long chain of competing and cooperative elements (Fedorchak, Kaminski, & Lammerding, 2014; D.-H. Kim et al., 2016; Wang et al., 2009). The mechanical transmission in this direction is coordinated by the physical interaction of integrins, actomyosin complex, LINC complex and lamins ending with chromatin compaction (Arsenovic et al., 2016; Osmanagic-Myers, Dechat, & Foisner, 2015). Even further, it seems that mechanical forces are transmitted inside the nucleus forming internal actin filaments (Chang et al., 2018; Le et al., 2016; Plessner, Melak, Chinchilla, Baarlink, & Grosse, 2015). If the cytoskeleton

dynamics seems well described in models, special efforts have been dedicated to study nuclear mechanotransduction in details, including the mechanisms underlying the application of force upon the nucleus via actomyosin (Arsenovic et al., 2016). Another important mechanotransduction mechanism impacting the cell that is relevant and needs to be considered is the nuclear envelope, as the force-dependent deformation of this membrane may cause the whole cell to change its shape and phenotype (Wang et al., 2009). The nuclear envelope is composed of the nuclear lamina proteins and other elements that attach the lamina to the nuclear membrane and connect it, through the perinuclear space, to the cytoskeleton (Gruenbaum, Margalit, Goldman, Shumaker, & Wilson, 2005). Therefore, when looking for the internal compositions, structures and dynamics of the nucleus that are responsible for its mechanical behavior, it was found that the particular properties and complex interactions between microtubules, myosin-II, lamin-B, lamin-A/C and heterochromatin cause nuclear tension and stiffness through their stoichiometric levels inside the cell and their intrinsic or collective mechanical properties (J.-K. Kim et al., 2017; Kirby & Lammerding, 2018). In fact, the importance of such mechanosensitive elements is such that many serious diseases like cancer, heart disease, muscular dystrophy and progeria have been linked to deficiencies or mutations of lamins, for instance (Ferrera et al., 2014). In general, lamin-A/C increases with the tissue apparent micro-stiffness or extracellular matrix stiffness *in vitro* while lamin-B levels remain low (Hadden et al., 2017; J. Swift et al., 2013; Joe Swift & Discher, 2014). Also, nucleus heterochromatin and lamin-A have been found to be responsible for the rapid transmission of mechanical cues of the external microenvironment of the cell and thus help deforming it and maintain the nucleus resistant to external stresses (Haase et al., 2015). Interestingly, it was later found that those proteins are responsible for two separate types of mechanotransduction processes: on one side chromatin, linked to the nuclear envelope through lamin-B receptors, governs only the response to small deformations; on the other side lamin-A/C controls the response to larger deformations (Stephens, Banigan, Adam, Goldman, & Marko, 2017). This existence of two regimes may be observed further in the anticorrelations between lamin-A/C protein and lamin-B receptors (Solovei et al., 2013). If the majority of the studies focus on a cyclic or reversible mechanotransduction phenomena of the cytoskeleton and look for the correlations between mechanical cues and signalling pathways responsible for cell responses in some cases, experimental data have identified irreversible behaviors that are intrinsically linked with nuclear deformation processes (Tocco et al., 2018). Besides the role of these well-known nuclear envelope proteins, an additional important regulation of the forces applied on the nucleus has been found in the rheology of the nuclear chromatin, as its deformable structure has found to be plastic and not isolated from the organization of the lamin-A/C network to distribute stress on the nucleus (Miroshnikova, Nava, & Wickström, 2017; Poh et al., 2012).

In order to analyze cell mechanobiology and thanks to all the experimental data material available today, efforts have been made to construct physico-mathematical models enabling the computation of cell size or shape evolution as well as spreading dynamics or migration as a function of cell environment stiffness. The physico-chemical models constructed result very useful in predicting the dynamic cell response to mechanical cues because the full mechanosensitive and stress-transmission chain is taken

into account, from the mechanical properties of the substrate to the internal nucleus through focal adhesion and the assembly-disassembly cycles of positive feedback actomyosin apparatus. Due to the complexity of all these mechanisms, modelling the nucleus as a mechanosensing organelle is not an easy task. Most models are looking at focal adhesion points, cell-cell unions and corresponding shapes and are thus limited to flat (2D) elastic matrices, useful to describe conventional *in vitro* experiments. Building on such pre-existing models and incorporating all, a more recent framework has managed to integrate the influence of known pathways and focal adhesion dynamics to build a multiscale model capable of predicting cell evolution on fibrous matrices (Cao et al., 2017). The same research group also managed to get closer to the full mechanosensing path from focal adhesions down to the nucleus by including actin contraction and cytosolic back-pressure responsible for the stress applied upon the nucleus of the cells (Cao et al., 2016). This resulted a much more realistic model to describe mechanotransduction effect in cell migration as chromatin was considered a plastic material. Another interesting model has been developed to successfully predict cell shape, contractility and polarization as a function of matrix stiffness using irreversible thermodynamics; it was able to describe durotaxis including contributions from several mechanotransduction pathways (Shenoy et al., 2016).

It is well known that cellular spreading is controlled by the mechanical characteristics of the substrates as this signal is directly transduced by coupling to the actomyosin complex as referred in the clutch model (Giannone, Mège, & Thoumine, 2009; Parsons, Horwitz, & Schwartz, 2010). In addition,, there are some experimental data that demonstrated that cellular anchoring is affected by alterations in nuclear lamins (laminopathies) (Bertrand et al., 2014; Hale et al., 2008). Loss of the interaction between lamin-A and emerin also affects cell spreading (Emerson et al., 2009). Surprisingly, depletion of type-A lamin in human fibroblasts directly affected the focal adhesion complex (FAC) assembly, nevertheless, an experimental rescue improved the phenotype. Moreover, the authors reported an impaired adhesive capacity that correlates with the loss in wound healing potential and impaired translocation of MRTF-A mechanotransduction cofactor (Corne et al., 2017). YAP activation is also altered in myoblast with mutations in lamin-A (Bertrand et al., 2014). During development, lamin-A is not expressed in mouse and human embryonic stem cells (ES) that express exclusively type-B lamins. In fact, MSCs are also deficients in lamin-A protein. Lamin A expression appears in the differentiation programs of different lineages (Röber, Weber, & Osborn, 1989); (Constantinescu, Gray, Sammak, Schatten, & Csoka, 2006). Cell reprogramming of MEF to pluripotent stem cells (iPS) is facilitated by downregulating lamin-A protein expression and, on the contrary, overexpression of lamin-A impaired reprogramming of iPS (Zuo et al., 2012). Interestingly, cytoplasmic components in ES have the potential to remove the deposition of lamin A protein in nucleus and this effect is an ATP dependent process (Bru et al., 2008; Zuo et al., 2012). It seems that pluripotency is highly related to the expression of lamin A and it is likely that pluripotency promoting genes (Oct4, Sox2, Klf-4 and c-Myc) are involved in this control.
Mechanical memory is also a very interesting behavior that has not been well understood (Kanoldt, Fischer, & Grashoff, 2018). It consists in priming cells to a subsequent mechanical stimulus. Usually, isolated cells are cultured in soft or stiff substrates during a specific period of time (passes) to establish

an adaptation to specific mechanical conditions, but then cells are challenged to respond to a different mechanical environment and often respond accordingly to their past conditions. It has been demonstrated that MSCs are primed to fibrotic phenotype when they are isolated and cultured on stiff substrates or 100 kPa (C. X. Li et al., 2017); this process depends on MRTF-A activation. Also, in MSCs osteogenic differentiation is primed and it is a YAP-dependent process (Yang, Tibbitt, Basta, & Anseth, 2014). Fibrotic primed phenotype is also observed in lung fibroblasts that retained the activated phenotype even cultured on soft substrates and myofibroblast activation is partially inhibited (Balestrini, Chaudhry, Sarrazy, Koehler, & Hinz, 2012). However, it is not congruent with the experiments obtained in liver hepatic stellate cells (HSC) where delay stiffening accelerated the myofibroblast transdifferentiation of these cells (Caliari, Perepelyuk, Cosgrove, et al., 2016). Besides differentiation, similar YAP-regulated mechanical memory effect related to irreversibility has also been observed in collective epithelial migration, where the long-term exposure to past matrix stiffness dictated the behavior of cells (Nasrollahi et al., 2017). Cellular memory is not only regulating final differentiation processes but also seems to have an important role in chronic fibrosis. It was demonstrated that HSC cells adapted from stiff substrates to soft conditions and challenged to a second stiff stimulus afterwards retained cellular memory from the stiff condition because comparing these cells with a cell maintained in soft substrates, cells challenged to a second mechanical stimulus responded faster and strongly (Caliari, Perepelyuk, Soulas, et al., 2016). It is also known that YAP is activated upon nuclear response to mechanical stretch, suggesting a correlation between the observed mechanical memory and mechanotransduction (Driscoll, Cosgrove, Heo, Shurden, & Mauck, 2015). Other interesting examples of mechanical memory altering mechanoresponse after pre-exposure to a different stiffness are liver stellate cells and lung fibroblasts (Balestrini et al., 2012; Friedman, Roll, Boyles, Arenson, & Bissell, 1989). Irreversibility is also considered in development, as it has been recently shown that the pluripotent embryonic stem cells are not mechanosensitive before exiting ground state (Verstreken, Labouesse, Agley & Chalut, 2019). Interestingly, the nuclear envelope proteins lamin-A,C are not involved in the early stages of embryonic stem cells differentiation (Ankam et al., 2018; Smith et al., 2017). It is finally interesting to remark that it has been impossible to induce pluripotent stem cells from differentiated cells without provoking a lamin-A deficiency prior to the induction; on the contrary, it has been shown that cells overexpressing lamin-A,C present slower and less efficient reprogramming (Zuo et al., 2012), that the nuclear lamin-A is mechanoresponsive to the extracellular matrix stiffness in mature tissues (Joe Swift & Discher, 2014) and that this protein helps shaping the cells to protect nucleus from stress (J.-K. Kim et al., 2017).

In order to study the relationship between internal and external mechanical signals as well as the mechanisms related to deformation, shape and dynamic behavior at the scale of nucleus and the whole cell, we performed a 1-dimensional analysis of the full mechanotransduction chain of the cell: nucleus, actomyosin apparatus and focal adhesion complex, directly connected to the extracellular matrix. We adapted and modified the existing mechanotransduction models detailed in (Cao et al., 2017, 2016; Shenoy et al., 2016) which successfully describe the growth of the focal adhesion complex of a cell as a

function of actomyosin forces and how both nuclear and extracellular matrix stiffnesses stabilizes the system. In this particular model of the the nuclear dynamics in transendothelial migration (Cao et al., 2016), the nucleus was considered an hyperelastic shell (the nuclear envelope) filled with a soft porous-elastic solid material (made of chromatin and subnuclear elements) but the relaxation of the nucleus due to its viscoelastic properties was not really taken into account considering the time range of interest in their temporal analysis. In order to include the time lapse before of the relaxed nucleus in our model, we used a Maxwell linear viscoelastic model with two arms where one branch represents the viscoelastic nature of the nuclear envelope and the second one represents the viscoelastic behavior of the subnuclear elements, mainly chromatin. As suggested by (Stephens et al., 2017), it is indeed crucial to disentangle and fully understand the individual role of nuclear chromatin and lamins because it might help better predict the deformations of cell nucleus induced by actomyosin-transmitted stress from the extracellular environment, and in particular the measurable apparent micro-stiffness of the adhesion substrate. Recent evidence has shown a plastic behavior of the nucleus, directly impacting the nuclear morphology and genes expression and the explanation of nuclear deformation probably lies in the viscoelastic properties of viscous lamin-A and elastic lamin-B. In this work, we propose that the dissipative nuclear deformation is a possible underlying mechanism behind mechanical memory (irreversible strain) and mechanical threshold in some pathologies like fibrosis. We believe this model is simpler to use as it only requires unsophisticated experimental measurements: conventional techniques enabling the application of a constant deformation upon the nucleus (such as AFM or nanoindentation) are sufficient to obtain stress versus time relaxation curves from which it is possible to reconstruct the cell and nuclear behaviors using only the lamin-A and lamin-B stoichiometric levels.

Materials and Methods.

Our model was built on a previously reported one that considered the full cellular chain of transmission of mechanical stresses from the adhesion substrate or matrix towards the nucleus, adding the viscoelastic properties of the nucleus that would be responsible for the dissipative behavior of the nucleus and may help better predict the cells morphological and phenotypic response as well as the mechanical memory that cells experienced when the stiffness of their environment is modified. A double Maxwell linear viscoelastic model was used to describe the nucleus (equation 1), as depicted in Figure 1. This model was proposed to simulate the viscoelasticity of nuclear envelope, principally made of lamin A, B and C, as experimental data show this behavior very clearly (J. Swift et al., 2013). It allows a more natural description of nuclear relaxation, reported in the order of seconds, and the nuclear plasticity. In order to describe this behavior, we then used the stress-strain relation of the dynamic element that represents the contractile force of actomyosin (equation 2) and of the effective stiffness describing the focal adhesion complex connected to a single fiber of extracellular matrix (equation 3). We related this constitutive equation to the viscoelastic behavior of the nucleus. The equations were manipulated in the Laplace space to simplify the mathematical treatment and solving. This is acceptable with the previous hypothesis that initial and boundary conditions are time independent or may be described by functions

whose components may be represented by the product of the time- and space domains. In the Laplace frequency space, we used the notation $L(x(t)) = \bar{x}$ where *L* is the Laplace transform operator. We thus obtained:

$$\bar{\sigma}_n = (\xi_{NE} + \xi_{ch})\bar{\varepsilon}_n \qquad (1)$$

$$\bar{\sigma} = \rho + k\,\bar{\varepsilon}_{ac} \qquad (2)$$

$$\bar{\sigma}_{FA} = k_{eff}\bar{\varepsilon}_{FA} \qquad (3)$$

where $\bar{\sigma}_n$ and $\bar{\sigma}_{FA}$ are the stresses applied upon the nucleus and focal adhesions respectively, mainly caused by the contractile stress $\bar{\sigma}$ associated with the deformations or strains of the nucleus $\bar{\varepsilon}_n$, the actomyosin contractile apparatus $\bar{\varepsilon}_{ac}$ and the focal adhesion complex $\bar{\varepsilon}_{FA}$. $\xi_{NE} = \frac{k_{LB}s}{s+\frac{1}{\tau_{NE}}}$ describes the mechanical properties of the nuclear envelope, where $\tau_{NE} = \frac{\eta}{k_{LB}}$, defined by the ratio of viscosity η associated to the behavior of Lamin A/C and the stiffness k_{LB} associated with elastic behavior of lamin-B (J. Swift et al., 2013). Similarly $\xi_{ch} = \frac{k_{ch}s}{s+\frac{1}{\tau_{ch}}}$ describes the mechanical properties of subnuclear components, mainly chromatin, where η_{ch} and k_{ch} are its viscosity and stiffness, with a corresponding characteristic time of $\tau_{ch} = \frac{\eta_{ch}}{k_{ch}}$. Equations 2 and 3 in the time-domain are detailed in (Cao et al., 2017, 2015; Shenoy et al., 2016). In equation 2, the term $\rho = \frac{\beta\rho_0}{(\beta-\alpha)s} + \frac{\alpha k-1}{\beta-\alpha}\varepsilon_{ac}$ describes an active contractile element with positive feedback: ρ_0 is the basal contractility of the cell in the absence of extreme stress or restriction (it may be interpreted as as the intrinsic contractile tension, or pre-stress); α and β are mechanochemical coupling parameters associated with a molecular mechanism reflecting the stress-dependent signaling pathways and engagement of motors respectively and they satisfy the conditions $0 < \frac{\alpha}{\beta} < 1$ (Shenoy et al., 2016); *k* represents the stiffness of cytoskeleton. Therefore, ρ and k describe the behavior of myosin motors in the elastic actin stress fibers. k_{eff} represents the effective stiffness transmitted to the cell by the focal adhesion complex described by (Cao et al., 2015) and which complete form and components are discussed in the Supplemental Material. It is a function that depends on the stiffness k_p of the adhesion plaque, the clutch k_c and substrate k_s and on the characteristic adhesion distance L_c and spacing between integrins d_c (Figure 1B). For simplicity, we decided to elaborate the model for a cell anchoring itself in a scaffold made of fibers. As the components of this model are connected in series, we have:

$$\bar{\sigma}_n = \bar{\sigma}_{FA} = \bar{\sigma} \qquad (4)$$

and by geometrical constraint we obtain:

$$\bar{\varepsilon}_n + \bar{\varepsilon}_{ct} + \bar{\varepsilon}_{FA} = 0 \qquad (5)$$

Solving equations 1 to 5 as a function of stress, it was possible to obtain the nuclear deformation as a function of time, obtaining equation 6 with the same form than that reported by (Cao et al., 2017) but in the frequency domain:

$$\bar{\sigma} = \frac{\beta\rho_0}{(\beta-\alpha)s}\left(\left(\frac{1}{\xi_{NE}+\xi_{ch}} + \frac{1}{k_{eff}}\right)\left(\frac{\alpha k-1}{\beta-\alpha}\right) + 1\right)^{-1} \qquad (6)$$

Moreover, using an inverse Laplace transform L^{-1}, it was possible to calculate the dynamic coefficients associated to the nucleus, and if we suppose a constant strain rate (or sudden stress) of a certain duration, it was possible to calculate both the relaxation and creep moduli. By using equations 1 and 6, it was possible to calculate the nuclear strain as a function of several parameters of interest, such as the substrate stiffness and the viscoelastic properties of the nucleus. For the latter, it was possible to calculate the reversible and irreversible deformations of the nucleus as a function of time when the nucleus is subjected to constant stress. From equation 1, $\bar{\varepsilon}_n$ may be considered constant and using L^{-1} we obtain:

$$\sigma(t) = (k_{LB} exp(-t/\tau_{NE}) + k_{ch} exp(-t/\tau_{ch}))\varepsilon_0 \quad (7)$$

considering that $cosh(x) + sinh(x) = exp(x)$:

$$\sigma(t) = [k_{LB}(cosh(-t/\tau_{NE}) + k_{ch}(cosh(-t/\tau_{ch})] + [k_{ch} sinh(-t/\tau_{ch}) + k_{LB} sinh(-t/\tau_{NE})]\varepsilon_0 \quad (8)$$

demonstrates that $\sigma(t) = (E_{store} + E_{loss})\varepsilon_0$ where:

$$E_{store}(t) = (cosh(-t/\tau_{NE}) + k_{ch}(cosh(-t/\tau_{ch}) \quad (9)$$

$$E_{loss}(t) = (sinh(-t/\tau_{NE}) + k_{ch}(sinh(-t/\tau_{ch}) \quad (10)$$

And in the frequency-domain they are described as:

$$E'(\omega) = \frac{\omega^2 k_{LB} \tau_{NE}}{\omega^2 \tau_{NE}^2 - 1} + \frac{\omega^2 k_{ch} \tau_{ch}}{\omega^2 \tau_{ch}^2 - 1} \quad (11)$$

$$E''(\omega) = \frac{\omega k_{LB} \tau_{NE}}{\omega^2 \tau_{NE}^2 - 1} + \frac{\omega k_{ch} \tau_{ch}}{\omega^2 \tau_{ch}^2 - 1} \quad (12)$$

Interestingly, equations 11 and 12 were derived from the initial model and are consistent with the literature for the dynamic moduli, as the total dynamic modulus is the sum of the dynamic modulus of each arm, as presented in (Jalocha, Constantinescu, & Neviere, 2015). These equations basically show that the dissipation of the elastic energy is directly proportional to the magnitude of viscous properties of both the nuclear envelope and the subnuclear elements. Finally, supposing a constant stress, we obtained the expression of the creep modulus:

$$Creep_n(s) = \frac{\sigma_0}{(\xi_{NE} + \xi_{ch})s} \quad (13)$$

To establish the time-domain form of equation 13 and calculate the other relations we used Wolfram Mathematica v11.3.

Code availability: the full model is shared as a notebook in Wolfram Mathematica and can be accessed here

Results.

First, we described our dissipative mechanical models of an adherent cell, considering the nucleus either as a viscoelastic material with the viscoelastic contributions η_{ch} and k_{ch} from the subnuclear elements (mainly chromatin) on one side, in parallel with η_{LA} and k_{LB} on the other side, representing the viscoelastic properties given by viscous lamin-A,C and elastic lamin-B respectively (Figure 2B). This model is compared with the current model (Figure 2A) that considers the nucleus only as an elastic

material of stiffness k_n. In both cases, the nucleus is connected in series to a contractile element ρ connected in parallel to the passive stiffness k of the stress fibers of the cytoskeleton in turn connected to a simple spring consisting in the apparent stiffness k_{eff} of the FAC connected to the ECM. As can be seen in Figure 2C for different tissues, the dissipative model accounts for the realistic behavior of viscous dissipation of some of the elastic energy transmitted to it by the nucleus. The nuclear strain was plotted in Figure 2C as a function of the ECM stiffness. The strain was normalized against the natural conditions of each tissue: levels of lamin-A,C and lamin-B and micro-stiffness as reported experimentally by (J. Swift et al., 2013), the values were calculated for a time of 10 seconds as this was considered the time of assembly/disassembly cycles of the stress fibers, reporting to be of between 10-30s, according to (Carvalho, Desai, & Oegema, 2009; Salbreux, Charras, & Paluch, 2012; Wilson et al., 2010). Despite the fact that both models describe a nuclear strain increase as a function of ECM stiffness augmentation (Figure 2C), there is a clear decrease of the slope of strain vs. ECM stiffness due to the viscous dissipation; a behavior in agreement with (Discher et al., 2005; J. Swift et al., 2013) but not found in the elastic model. It is interesting to note that soft phenotypes (such as liver and kidney, for instance) present a greater nuclear strain than stiffer ones (muscle and heart). The results also imply that there is a lower dissipation of the elastic energy exerted by cells coming from softer tissues in order to limit their nuclear deformation when their ECM stiffness increases. This coincides with a lower lamin-A,C proportion in cells constituting soft tissues, resulting in a lower viscosity, hence dissipation.

Then, with the help of the equations described in the previous section and actual experimental values (Table S1 of the Supplemental Material), nuclear strain density maps were drawn for three selected tissues (soft liver, intermediate lung and stiff heart) in order to understand the frequential nuclear deformation for different ECM stiffnesses as a function of the intrinsic properties of the nucleus of the cell. We remind here that the cell type is linked with the lamin-A and lamin-B proportion of the nuclear envelope. Figure 3A demonstrates that the frequential evolution of the nuclear strain is very much dependent on the nuclear mechanical properties at low frequencies (we selected the 0 - 300 mHz range because of the 10-30s stress fibers cycles mentioned above). It is visible that softer tissues cells suffer more deformation as the frequency increases, a sign of a lower dissipation correlated to a less viscous behavior caused by the lack of lamin-A,C: the deformation of cardiac tissue cells on a 10 pN/nm ECM stiffness is two third less than a liver cell and 50% less than a lung cell on the same substrate. Due to the temporal nature of the dissipative model used here (Figure 2B) it is possible to know the dynamic creep and relaxation moduli, presented in Figure 3B and 3C respectively. These moduli are also function of the particular properties of the cell type (intrinsic nuclear mechanical properties dictated by lamin-A-C and lamin-B levels). These dynamic creep/relaxation moduli are useful to describe the effect of a constant stress/deformation imposed by external stimuli on the nucleus of the cell. The presented behaviors shown here correspond to what has been reported in different works (Chaudhuri et al., 2016; Pajerowski, Dahl, Zhong, Sammak, & Discher, 2007; J. Swift et al., 2013). Being able to calculate and analyze the two moduli separately is a characteristics of materials which present a linear viscoelastic behavior; this allowed us to identify and discriminate between the reversible and irreversible part of the

nuclear deformation and analyze potential irreversible phenomena that were observed experimentally as mentioned in the introduction. Without loss of generality we used the creep modulus to represent this phenomena in Figure 3D, where we first calculated the time-constant reversible part of the modulus corresponding to creep(t=0) and the irreversible part (bold colored line) calculated as creep(t) - creep(t=0) for the three tissues used in Figure 3A. The intersection of both parts mathematically happens for $t = \tau = \frac{\eta_{LA}}{k_{LB}}$, implying that the limit of reversible deformation of the nucleus under a constant stress has two components, one mechanical and one temporal. This means in turn that a sudden stress applied on the nucleus under the creep limit during a time $t < \tau$ will not provoke irreversible deformations while the same stress for a longer period of time $t > \tau$ will provoke irreversible deformations of the nuclear morphology. It is also very important to remark that the characteristic time τ is only dependent on subnuclear viscosity and elasticity given by the lamin-A.C and lamin-B levels, as suggested and later shown by Discher and colleagues in several papers. Therefore, for tissues presenting a greater τ , called “stiff phenotypes”, the contribution of the reversible part is greater than that of the irreversible one, suggesting a more stable phenotype on stiff ECMs. Finally, it is important to note that knowing these dynamic moduli help establishing the conditions under which the nuclear deformations will turn plastic, impeding the nucleus to return to its initial morphology without additional signals that would modify its mechanical properties.

To test the developed model further, the contractile stress (Figure 4A) and nuclear strain (Figure 4B) were calculated as a function of the ECM (or substrate) stiffness for different nuclear viscosities and stiffnesses. Equation 6 was used to calculate the contractile stress while strain was obtained from substituting equation 6 in equation 1. The results are shown in Figure 4B for different ECM stiffnesses of 0.5, 1, 5 and 40 pN/nm. Both stress and strain were normalized against intrinsic contractile stress and strain and the values presented here were calculated for s=0.1, equivalent to a 10s time. Indeed, even though a cell is in a stable state, described by equation 5, maintaining a fixed morphology (either extended or compact) its nucleus is still stressed during a certain amount of time, that depends on the contractile cycles of actomyosin. This may result in a temporal or permanent deformation that is very much dependent on the mechanical properties of the nucleus. Our proposed model for the nucleus (depicted in Figure 2B and equation 1 and 6) shows that in spite of an increase in stress due to an increase in extracellular substrate stiffness, the nucleus presents a dissipated stress that depends principally on the proportion between the magnitude of the viscosity η_{LA} and the stiffness k_{LB} of the nuclear envelope. Equation 6, plotted in Figure 4A, exhibits an increase in the contractile stress due to an increase of the ECM stiffness, nuclear viscosity or stiffness, in perfect agreement with what was reported in the literature (Ghibaudo et al., 2008; Mitrossilis et al., 2009).
In Figure 4C, the particular example of the liver was taken to try to describe the evolution of the nucleus strain as a function of the evolution of ECM stiffness that fibrosis causes in the tissue. We plotted the predicted curves of nuclear strain as a function of the viscoelastic properties of the nucleus for different micro-stiffnesses: experimental data show that a 1.2pN/nm corresponds to a normal (soft) tissue while

12pN/nm is the fibrotic tissue threshold and 22pN/nm is completely fibrotic (Castéra et al., 2005; Desai et al., 2016; Mueller, 2010). According to the work of Discher and colleagues, η_{LA} and k_{LB} can be inferred for normal liver cells and these coordinates were positioned on the density map at the corresponding stiffness of 1.2pN/nm (blue spot). As stiffness increases (next density plots), it has been proposed that the lamins concentrations given by [lamin-A] ~ $E^{0.7}$ and [lamin-B] ~ $E^{0.2}$ need to be gradually increased to respond to the gradual stress, and thus reduce the strain caused by the ECM stiffness increase. Therefore, we have calculated and placed new positions corresponding to lamin-A,C and lamin-B levels that make sense physically and are suggested by Discher and colleagues in their lamin-A:lamin-B vs. micro-stiffness plots at 12 and 22 pN/nm. This enables us to better show that our model seems to explain that the increase of lamins while maintaining the proper ratio as a function of micro-stiffness (presented by Discher) may be a direct consequence of stiffening directed at reducing the nucleus strain when the substrate is stiffening (dark blue regions in the density plot). It is then apparent that, when the ECM returns to softer stiffness values, the nucleus may remain in its previous stable state of high lamins levels acquired during stiffening, as it is not requiring any further modification of its mechanical properties to reduce strain (see dotted arrows). Our model might then explain the hysteresis behavior of the mechanical memory found experimentally in soft-to-stiff-to-soft cycles (Balestrini et al., 2012; Caliari, Perepelyuk, Soulas, et al., 2016; Maeshima, Tamura, & Shimamoto, 2018; Nasrollahi et al., 2017), where several types of cells transitioned from soft to stiff substrates will evolve more rapidly if they have already been in contact with stiffer conditions prior to that. This particular point is of great interest and will be discussed further in the following section.

Finally, due to the dynamic nature of our model, that describes explicitly the temporal evolution of nuclear strain, it was possible to to calculate the magnitude of the velocity and acceleration of nuclear strain for different tissues (corresponding to different η_{LA} and k_{LB} or vice versa) as functions of the effective stiffness of the ECM k_s . The results, obtained by successive derivatives of the strain as a function of frequency (s) and using an inverse Laplace transform achieved successfully with Wolfram Mathematica, are shown in Figure 5 and present the magnitude of velocity and acceleration of nuclear strain as a function of time. This model of the cell nuclear response of the apparent micro-stiffness of the substrate seems to suggest a possible spreading rate of the nucleus dependent on a contractile stress sustained for a certain period of time (in the order of seconds) and on the effective stiffness created by the FAC-ECM union. Figure 5 demonstrates a decrease of the magnitude of the velocity in “stiff phenotypes” (more lamin-A) and the model is predicting a non-linear behavior of the nuclear strain velocity. To the best of our knowledge, the nuclear dissipative model presented here might offer an excellent tool to start modelling nuclear deformation and establish the bases to define nuclear stability as suggested by Figure 5C where long-term acceleration (t = 1800s) reached a convergence value independent on nuclear envelope and ECM stiffness, thus phenotype. These larger periods of time may represent the drag of the nucleus by the actomyosin apparatus that occurs in cellular migration on 2D substrates. This nonlinear behavior and the stabilization of the nuclear deformation acceleration to a convergence value regardless of the ECM-stiffness or the tissue type suggest that it is of critical

relevance to account for the time period during which the nuclear deformation is actually happening. Depending on the cellular process under study, namely short-time stress causing nuclear strain due to ECM-stiffness or long-time cell migration, the nucleus will clearly respond differently. It is however interesting to note that the instantaneous velocity values of approximately 1μm/min found with our model after 60s for softer tissues are in good agreement with the range of experimental nuclear drag values reported very recently in similar conditions for neuroepithelia (Yanakieva, Erzberger, Matejcic, Modes, & Norden, 2019).

Discussion.

It is well known that adherent cells perceive, interpret and respond to mechanical stimuli using multiple mechanisms. Moreover, cells response to mechanics is not only dependent on external stimuli such as extracellular matrix stiffness or viscosity but is also affected strongly by its own capacity to modify its internal mechanical properties (Figure 1) and the rate of adaptation mechanisms (Cao et al., 2017). In particular, the cell nucleus is the subcellular component with the greatest stiffness in the cell (Caille, Thoumine, Tardy, & Meister, 2002; Lammerding, 2011)) and it has been reported that its intrinsic mechanical properties are as important as those of the ECM in stabilizing the contractile stresses of the cytoskeleton (Wang et al., 2009). Principally, the chain transmitting the stress to the nucleus (Figure 1B) is formed by the focal adhesion complex (FAC) linked to the actomyosin apparatus (the major source of contractile stress) connected to the nucleus by the LINC complex, thus uniting the cytoskeleton to the nucleoskeleton as depicted in Figure 1C. It is important to model the nucleus and integrate its mechanical behavior in a more complete mechanotransduction chain from the ECM-FAC unions to this organelle, because this could help understand and better control the nucleus morphology involved in genes expressions responsible of several pathologies or phenotype issues *in vitro*. The pre-existing descriptions and models used to build our present model of the cell consider the observed irreversibility of nuclear deformation but only consider an immediate transmission of the contractile stress to the nuclear envelope, which is not instantaneous in reality. For this reason, it is believed that the dissipative model presented here is an important contribution to those models as we have considered the dissipation caused by the viscoelastic nature of the nucleus that derives from two important sources: lamin type A/C and chromatin organization (Maeshima et al., 2018). This seemed to be a very important factor to consider in mechanotransduction and needed to be addressed in order to understand uncovered cell responses such as the mechanical memory of cells.

From an experimental point of view, it is important to establish a model that could relate directly to observed behaviors and quantifiable quantities, ideally in a simple way and obtaining the data using relatively simple techniques as suggested by the excellent and pioneering work of Discher and colleagues. Therefore, modelling the nucleus with two Maxwell systems in parallel may result very useful as the dynamic moduli *E'* and *E"* can be calculated in both the time domain (equations 9 and 10) or the frequency domain (equations 11 y 12). Moreover, the model allows a separate treatment of elastic and plastic contributions. In Figure 3B, we calculated the creep modulus and the relaxed modulus for

different types of tissues, using equation 13 and the relaxation modulus using equation 7. The characteristic time of each tissue was calculated using the $\tau = (Lamin\ A : B)^{2.5}$ relation, as proposed by (J. Swift et al., 2013; Joe Swift & Discher, 2014). The creep modulus is relevant experimentally for our particular problem, because when a sustained stress is applied for a given time upon a viscoelastic material, the system responds by increasing its deformation for a certain period of time, characteristic of the cell type. From this model of dynamic modulus, it is then possible to extract the data describing the elastic and plastic deformations of the nucleus. Figure 3B indeed enables the extraction of and comparison between the reversible and irreversible components of the fluence modulus and the prediction of (τ, *creep(t=0)*) pairs from which the material will suffer an irreversible deformation due to the viscoelastic dissipation. This corresponds to the point of inflexion for each tissue in Figure 2C, at constant stress. Moreover, the graphs of Figure 2C also show that the slope of the dissipated energy tends to the reversible curve in an asymptotic manner when $k_s \rightarrow \infty$. This information is of particular importance as the integration of the dissipative components in our model made the calculation of the threshold of irreversible elastic modulus possible. In the particular case of the liver shown in Figure 3D, the calculations of the irreversible elastic modulus threshold gave a value of 1.36 kPa (the inverse of the creep modulus of 0.73 kPa^{-1}), which is in excellent agreement with experimental data shown in (Desai et al., 2016; Stephens et al., 2017) where this particular matrix stiffness was the measured mechanical threshold from which the hepatocytes had lost their functions through activation of mechanotransduction pathways including Rho/ROCK signaling that reinforce actomyosin-based forces (Desai et al., 2016). This value is also commonly reported as the stiffness of fibrotic liver tissues, between 1.2 and 1.6 kPa (Georges et al., 2007; Yeh et al., 2002). This thus suggests that the lamin-A:lamin-B ratio used to determine the threshold in our model may also be involved in fibrosis pathologies.
In Figure 4B, it is suggested that nuclear strain obtained by stiffening of the ECM modified the ratio of lamin-A:lamin-B in response to mechanical conditions. Importantly, it is likely that the softening of the same ECM must be accompanied by the recovery of previous lamin-A:lamin-B ratio, however, the removal of lamin-A in the nuclear membrane depends on autophagic processes and it could limit a fast recovery to the initial state as autophagy depends on membrane trafficking and lysosomal activation (Dou et al., 2015; Lenain, Gusyatiner, Douma, van den Broek, & Peeper, 2015). Additionally to the fibrotic processes in the liver, differentiation and memory are closely related to nuclear strain caused by mechanical forces. It has been shown that the ratio of lamin-A:lamin-B increases with differentiation state and deficiencies in lamin-A affects differentiation in MSCs (Hadden et al., 2017; W. Li et al., 2011; Mateos et al., 2013; Perepelina et al., 2018).

Nuclear strain obtained at different stiffness shown in Figure 4C may explain mechanical cell memory observed in certain cell types; however, there are new data that sustain that cell memory process also acts through chromatin condensation caused by stress. Epigenetic modifications are promoted by nuclear strain and this stretch-induced strain increases chromatin compaction (heterochromatin) causing lineage commitment or cell memory in MSCs. In this work, gene silencing is regulated by polycomb pathway that is activated by emerin and non-muscle myosin IIA (Le et al., 2016). Lamin-A mechanical

transmission is downstream of emerin and non-muscle myosin IIA so activation of polycomb pathway and mechanical memory may be regulated by lamin-A-dependent nuclear strain. In another work, heterochromatin is increased by dynamic loading in nuclear strain and it is accompanied by accumulation of lamin-A in the nuclear envelope relating directly cell memory with lamin-A increase in nuclear membrane. Interestingly, authors demonstrated that cell commitment is blocked by TSA, an inhibitor of histone deacetylases (Heo et al., 2016, 2015). It suggests strongly that lamin-A deposition and heterochromatin formation are components of cell memory. Not only mechanical loading (stretch) controls cell memory, MSCs and fibroblasts cultured in a specific mechanical conditions are primed or conditioned to a committed phenotype or an enhanced response, respectively (Balestrini et al., 2012; C. X. Li et al., 2017; Yang et al., 2014)(C. X. Li et al., 2017; Nasrollahi et al., 2017)(Balestrini et al., 2012; C. X. Li et al., 2017; Yang et al., 2014). Unfortunately, in all these works there is not a direct relation between cell memory and lamin-A:lamin-B ratio modified by nuclear strain, however, it has been demonstrated that YAP and MRTF-A cofactors participe in the memory process; transcriptional cofactors whose activation correlates with nuclear strain (C. X. Li et al., 2017; Nasrollahi et al., 2017). (Hadden et al., 2017) demonstrated that cell differentiation correlates with lamin-A increase in nuclear membrane and YAP and MRTF-A activation but did not show cell priming. Another experimental data that suggest that cellular differentiation and memory are components of mechanical transmission and transduction from ECM stiffness to nuclear deformation and actin-dependent heterochromatin formation to control gene regulation is done by (Chang et al., 2018). In this study, the authors demonstrated that SWI/SNF complex inhibits YAP-TEAD transcriptional activation in soft substrates but in stiff conditions SWI/SNF complex is associated to nuclear actin filaments to rearrange chromatin and free YAP-TEAD complex to activate specific genes related to differentiation. Again, experimental data correlates global nuclear changes with chromatin remodelling but lacks of the direct link between nuclear strain and cell memory. All these data, suggest clearly that mechanical cell memory depends on nuclear mechanics and signaling downstream but a direct link between cell memory and lamin-A:lamin-B ratio has not been demonstrated. It is necessary to do experimental work that prove our dissipative model and help biologists to set values to mechanical irreversibility in order to correlate mechanical changes outside the cells to inner changes that dictates cell priming or memory. Experimental data are also needed to correlate lamin-A removal with cell plasticity and commitment influenced by mechanical environments. Lamin-A turnover is a process that depends on ubiquitination and autophagic-lysosomal pathways (Borroni et al., 2018; Lenain et al., 2015) and it is important to analyse the influence of lamin-A stability in cell memory.

In summary, our model may be a very useful tool to help understand nuclear response to external stress. By including the viscoelastic properties of the nucleus for the first time, it enabled the modelling of temporal evolution of both nuclear contractile stress and strain in a more natural manner, and it also was possible to reconstruct other results that were already reported by other groups using different models. Moreover, being able to calculate the energy dissipated by the nucleus caused by its viscoelastic nature (dictated by the lamin-A and lamin-B levels mentioned by Discher in his work), it is

possible to infer the temporal evolution of the nucleus morphology and consider it as a control and stability parameter, that may explain the mechanical memory that cells have shown to exhibit in several experiments. We believe that even though it is known that chromatin supports up to 30% of the nuclear strain, it is both the dissipation from the nuclear envelope and the chromatin that stabilize the strain and deformation; indeed, experimentally there is a clear relationship between the stoichiometric levels of viscous lamin-A, elastic lamin-B, ECM micro-stiffness and nuclear viscoelastic characteristics. The model also permit to infer that nucleus plasticity, linked to cell mechanical memory or cell mechanical history, only occurs at longer times compared with the irreversibility thresholds determined for each cell type, Figure 4C. The dissipative model presented here suggests to look further at the kinetics and evolution of the nuclear strain in diseases where the ECM stiffness is responsible for nuclear plasticity, as this could be induced by an increase of contractile stress causing a loss of stabilization and thus a loss of elasticity. Our results also suggest a convergence of the magnitude of nuclear strain velocity and acceleration when $\{t, k_s\} \rightarrow \infty$. This result is also observed in the frequency domain (Figure S1), implying an intrinsic behavior of the cell, because it does not depend on the domain where it is represented. The model may thus help establish criteria to study nuclear mechanical responses as it is clear that the timescale is very relevant. After a certain period of time (of approximately 30 min according to our model), the nucleus will deform at a fixed acceleration, regardless of the tissue type and ECM-stiffness. Clearly, this is not applicable to any cellular process: depending on the phenomenon observed or under study, for instance either instantaneous nuclear spreading or migration, the influence of the duration of stress upon the nucleus will have to be addressed. As actomyosin cycles are known to be on the order of 10 to 30 seconds, but in the case of cell migration, a process that ranges in the order of dozens of minutes, this is extremely relevant. The model integrating the viscoelastic property of the nucleus caused by its intrinsic elements and envelope managed to recapitulate the important work of Discher and colleagues over the years, where there is a clear contribution of lamin-A,C and lamin-B to the nuclear strain and stress under the contractile stress originated by the FA-ECM and transmitted to the nucleus. It also suggests to look closer at the nuclear lamins concentration levels in cyclic soft/stiff experiments (especially if stiff-to-soft progression) to try to understand better cell mechanical memory. And finally, the model seems to point towards a need to better measure and quantify the kinetics of nuclear strain experimentally, in order to better understand ECM-stiffness-related mechanisms that affect the cell nucleus. Indeed, the results obtained on the convergence of the acceleration of nuclear strain at large timescales demonstrate that the timescale during which stress is applied is of great relevance in defining the future of a cell. Using the same idea of a characteristic time for each cell type (but defined by Discher as the ratio of viscous lamin-A,C and lamin-B) the results could even open the discussion regarding the plausible hypothesis of a time-dependent phenotype, as it is clear that different cell types coexist on the same ECM stiffness but are not impacted in the same way. Of course it is not thought that only the mechanical properties of the nuclear envelope are responsible for such complex mechanisms and mechanotransduction pathways, and other factors intervening in the processes, such as the LINC complex, should be studied as they may modify the contractile stress transmitted to the nucleus, hence its deformability.

Finally, our model is only unidimensional to simplify the calculations but it predicts scalar quantities like stress time and magnitudes and is a very useful tool that enables the development of tensorial analysis of a greater order in the future for a more detailed and ascertained model in 3D, which should not be too complicated to build using this basis.

Supplemental Material.
Supplemental material presents more details on the calculation of the effective stiffness of the model, all the parameters and their corresponding values used in this work (with references) in Table S1 and an additional figure S1 on frequential velocity.

Acknowledgments.
Authors acknowledge DGAPA-PAPIIT projects number IT102917 and IA206818, CONACyT project #272894 for funding and Laboratorio Nacional de Soluciones Biomiméticas para Diagnóstico y Terapia (LaNSBioDyT) for support. We also thank UNAM for permitting using the license of Wolfram Mathematica. This article was submitted as a partial fulfillment of DPC's PhD Thesis and he acknowledges the Doctorate Programme in Materials Science and Engineering (Posgrado en Ciencia e Ingeniería de Materiales)

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All three authors DPC, GVV and MH are co-first authors of this manuscript.

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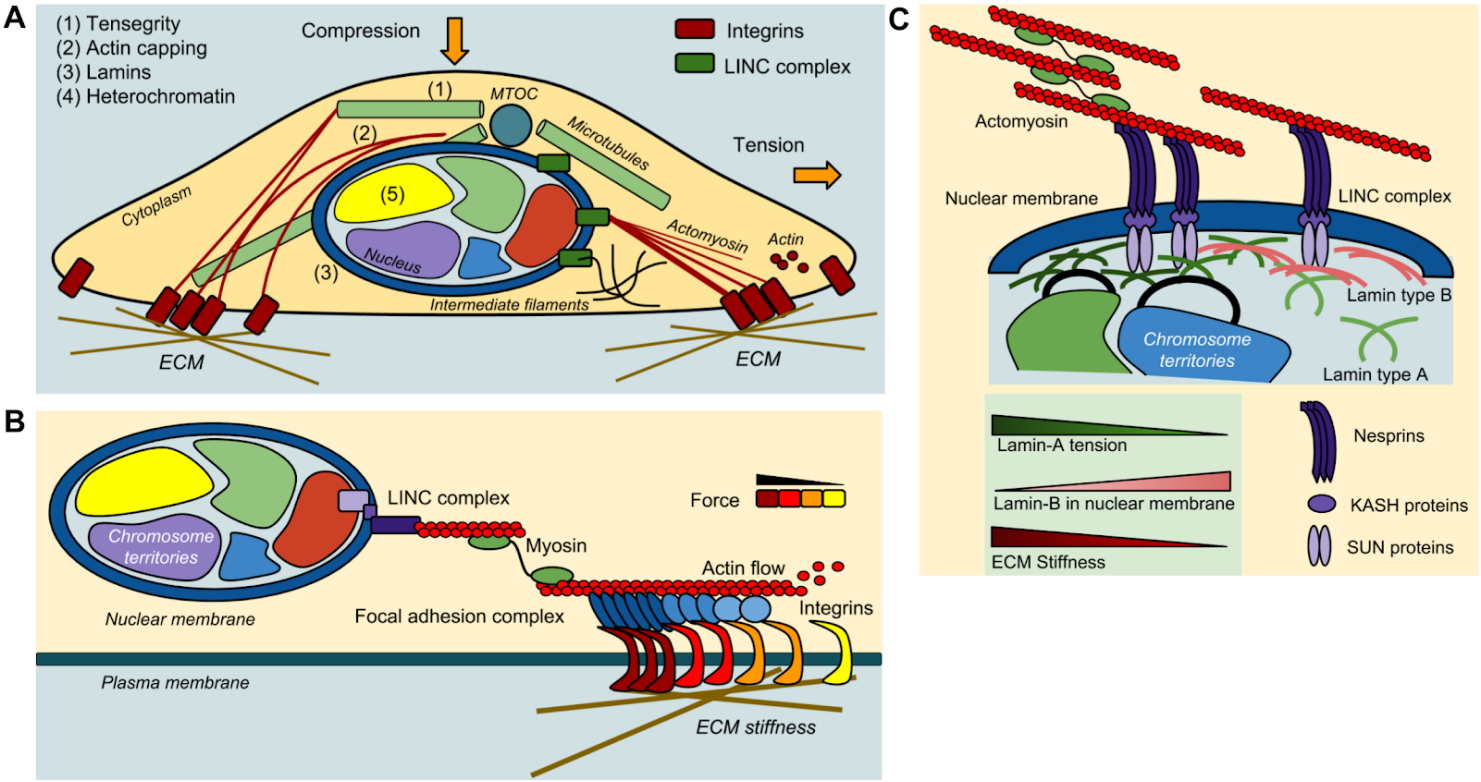

Figure 1. **Mechanical inputs that regulates nuclear elasticity.**
(A) Extracellular matrix (ECM) stiffness is sensed by heterodimeric transmembrane proteins (integrins) that interacts directly to ECM fibers and transduce the signal into the cell by coupling actomyosin contraction complex that establish the ECM-Integrins complex and transmits stress to nuclear transmembrane proteins. Actomyosin stresses and strains the nucleus by anchoring itself by LINC complex. Actin capping interacts directly to nuclear envelop to restrain nuclear deformation promoted by actomyosin and tensegrity compression forces. Nuclear cytoskeleton confers directly the mechanical properties of nucleus going from linear to nonlinear elasticity. Chromatin inside the nucleus also contributes to establish the mechanical characteristics of the nucleus through its intrinsic properties of highly compaction of nucleosomes and rearrangements promoted by epigenetic factors. **(B)** Integrins heterodimeric complexes in plasma membrane anchored to ECM proteins. ECM proteins cross-linking and composition confers the elastic properties of the ECM. This properties are transduced by the unfolding and rearrangement of proteins in the focal adhesion complex. Proteins into this complex such as talin and vinculin transduce the elastic properties of ECM into mechanochemical signals that begin in the establishment of actomyosin complex through the focal adhesion complex that confers a retrograde flow and transmits tension forces to nucleus. The connection between actomyosin contraction complex and nuclear envelope is carried out by LINC complex proteins that transmit the stress initiated by focal adhesions deforming the nucleus as a final result. **(C)** Nuclear deformation is carried out by actomyosin contraction and this tension is transmitted by LINC complex that is coupled to lamins inside the nucleus next to nuclear membrane. LINC complex is composed of nesprins that are linked to SUN proteins by NASH proteins. SUN proteins transmit stress from actomyosin to lamin type B and A and this stress

promotes deposition and unfolding of lamin type A. Deposition of lamin A and the ratio of lamin A/B modifies elasticity of nucleus from elastic to viscoelastic behavior. Lamins possess the ability to interact with DNA sequences and this interactions open or close large genome territories.

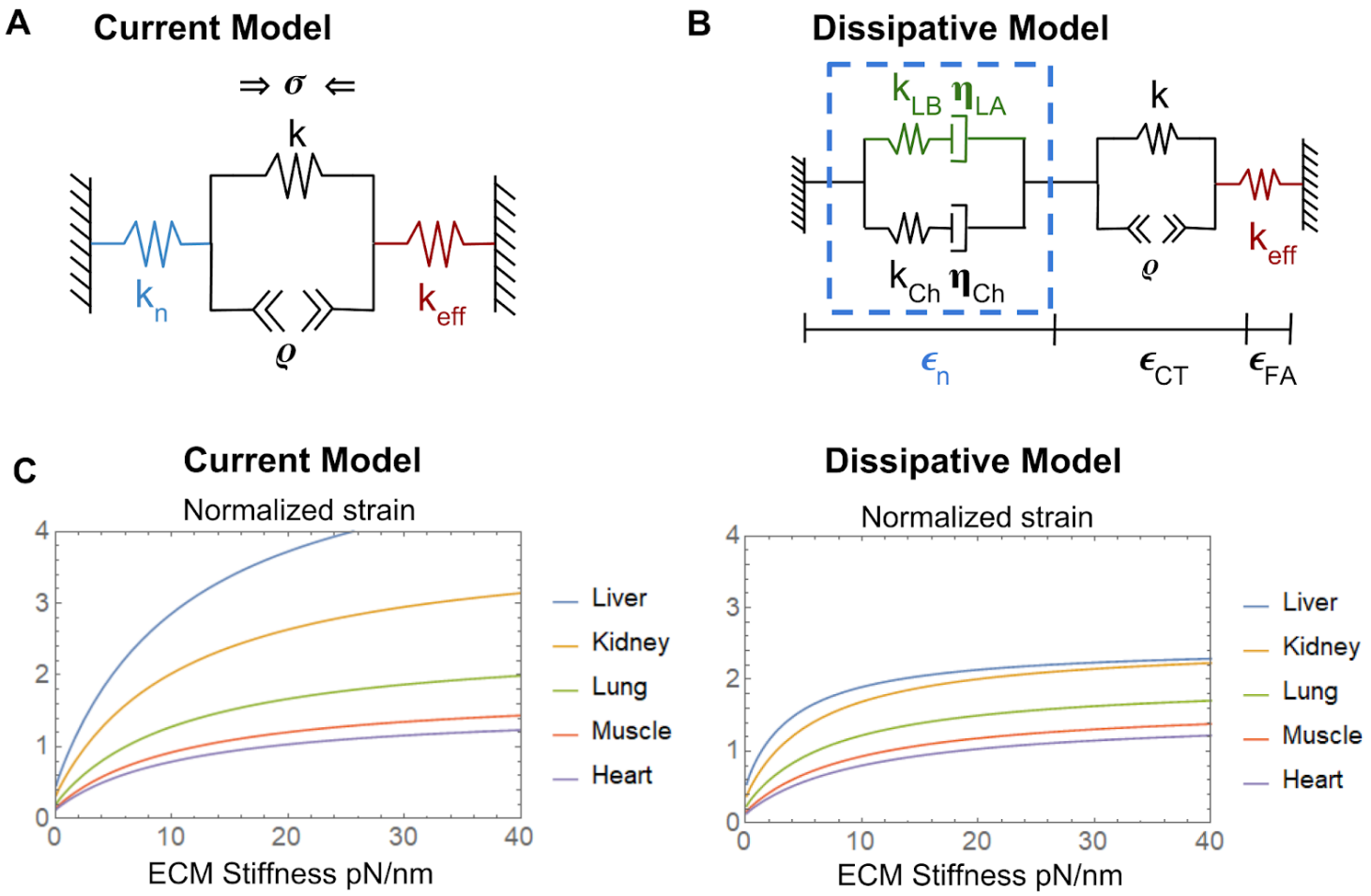

Figure 2. Dissipative mechanical model of the nucleus.

Two mechanical models of an adherent cell are presented, from the FAC to the nucleus and through the actomyosin apparatus responsible for the contractile stress. **(A)** In the existing model, the nucleus is considered an elastic material of stiffness k_n. **(B)** In our dissipative model, it is described as a viscoelastic material with the viscoelastic contributions η_{ch} and k_{ch} from the subnuclear elements (mainly chromatin) on one side, in parallel with η_{LA} and k_{LB} representing the viscoelastic properties given by viscous lamin-A,C and elastic lamin-B respectively. The difference between the two models lies in the dissipation of some of the elastic energy by the viscous elements as depicted in **(C)** where the nuclear strain of different tissues was plotted as a function of the ECM stiffness after 10s of sustained stress. The strain was normalized against the natural intrinsic conditions of each tissue. Viscous dissipation is visible in the dissipative model in the form of a decrease of the slope of the strain vs. ECM apparent stiffness for all tissues.

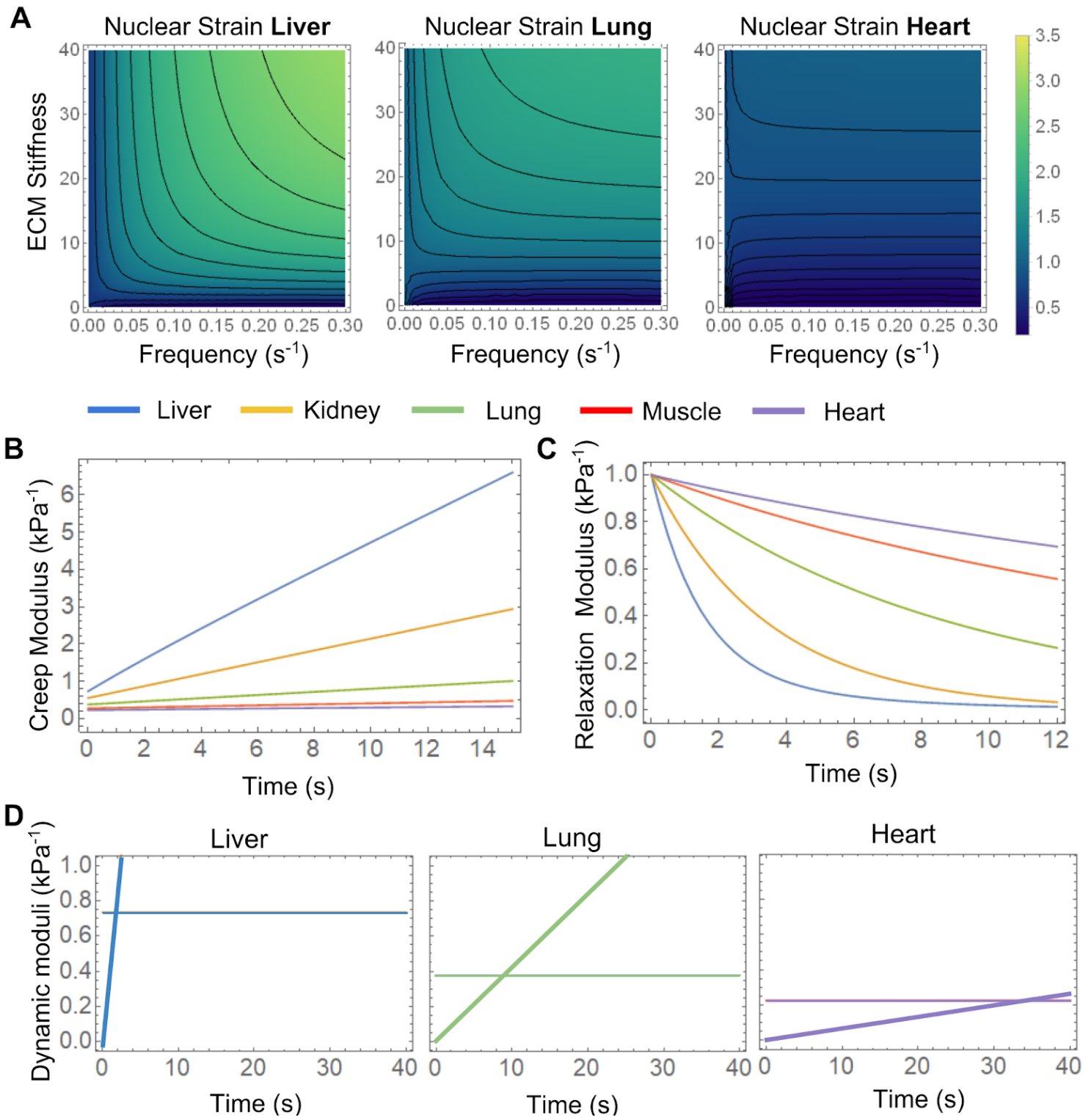

Figure 3. **Reversibility and irreversibility due to dissipation.**

The dynamic behavior of nuclear mechanical properties are described in this figure. **(A)** Nuclear strain density maps (normalized against the intrinsic condition) as a function of the frequency of stress and ECM stiffness for liver (soft), lung (intermediate) and heart (stiff) tissues. We observe that for a stiffness

lower than 10pN/nm, nuclear strain of the liver cells is twice as large as that of the lung and three times as large as that of the heart. **(B)** The creep and relaxation moduli were also calculated for different values of micro-stiffnesses of the same tissues ranging from soft to stiff. We calculated lamin-A and lamin-B concentration levels of each micro-stiffness using [lamin-A] ~ $E^{0.7}$ and [lamin-B] ~ $E^{0.2}$ as well as the characteristic time $\tau = (\frac{[Lamin-A]}{[Lamin-B]})^{2.5}$ (J. Swift et al., 2013). **(C)** The reversible (elastic) and irreversible parts of the creep modulus were calculated as creep(t=0) and creep(t)-creep(t=0) respectively and plotted as a function of time for three tissues. In particular, it was possible to identify the viscous contribution of the creep modulus under a constant stress. The slope of the irreversible part (bold line) decays as the stiffness of the tissue increases (lamin-A levels are more important in stiff phenotypes). These dissipative phenomena associated to nuclear plasticity might be the underlying mechanism of cell mechanical memory: after the intersection between reversible and irreversible components of the creep modulus, the nucleus is not able to go back to its original morphology.

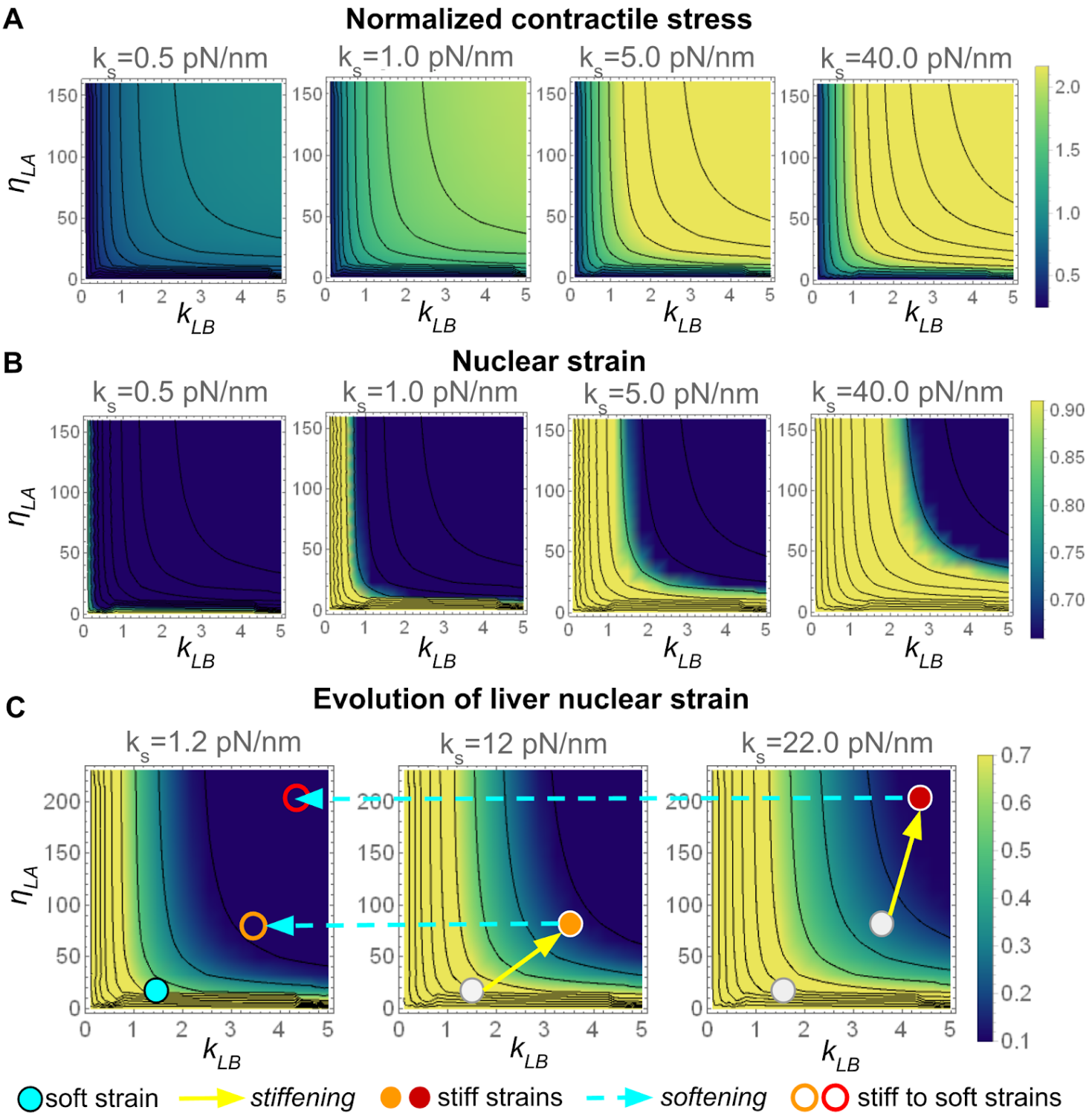

Figure 4. Nuclear stress and strain density maps

Thanks to equations 1 and 6, it was possible to compute **(A)** the nuclear stress and **(B)** nuclear strain density maps for different stiffnesses of the substrates (effective stiffness of the FA-ECM: k_s) and as a function of the magnitude of viscosity of lamin-A and stiffness of lamin-B η_{LA} and k_{LB}. Both were

normalized to the maximal stress/strain value. (A) shows the expected behavior that not only stress but also the magnitudes of the mechanical viscoelastic properties of the nucleus increase as k_s increases. (B) shows that there is a certain stabilization of nuclear strain (deformation) that depends strongly on the intrinsic values of η_{LA} and k_{LB} even when FA-ECM increases. Finally, **(C)** shows an example of a strain density map for the liver, using specific k_s that were reported in the literature (see Table S1). The purpose of these graphs is to show the direct application of our method to determine nuclear strain as a function of ECM stiffness and the mechanical properties of the nucleus (given by lamin-A and lamin-B concentrations). Using actual values reported in (J. Swift et al., 2013) and converting them into positions on these density maps, it is possible to observe that nuclear strain caused by an increase of the ECM stiffness (yellow arrows) is limited by a remodeling of the nuclear mechanical properties, described as an increase of lamins concentrations by (J. Swift et al., 2013) and also directly proportional to the mechanical properties used here ($\eta_{LA} = E^{1.75}$ and $k_{LB} = E^{0.5}$). However, when ECM is decreased for a stiff phenotype with higher lamins levels, the nucleus is already in a stable state without strain and no remodeling of its mechanical properties is required (blue arrows).

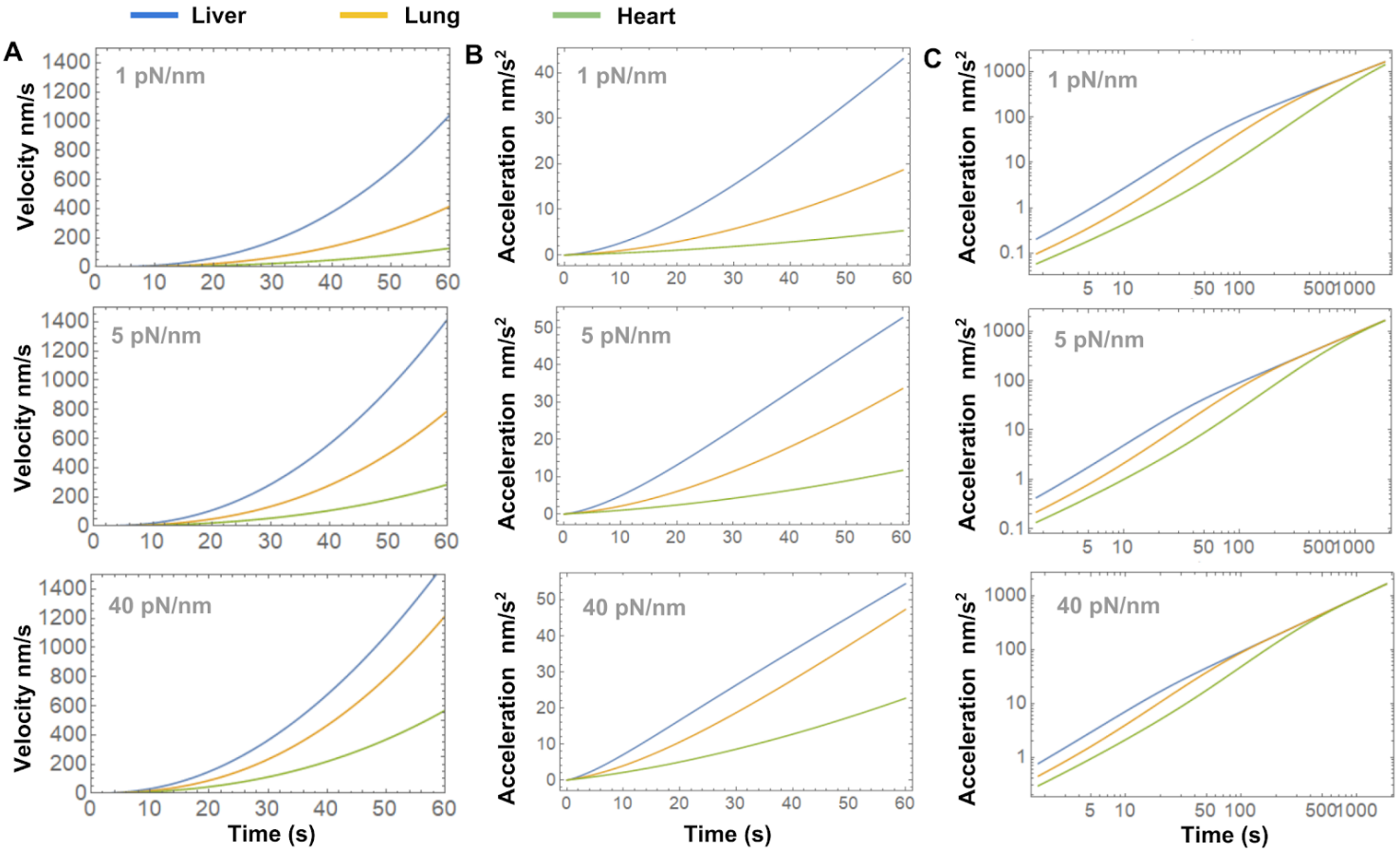

Figure 5. Nuclear spreading: velocity and acceleration of the nuclear strain.

Due to the dynamic nature of the model employed here (equation 1), it was possible to derive and plot the curves of the magnitude of the velocity and acceleration of the nuclear strain by successive derivatives of the strain as a function of the "s" parameter. This phenomenon may be related to the nuclear spreading or the drag caused by the stress fibers in cellular migration. Both originate in the contractile stress of myosin molecular motors. **(A)** Evolution of the magnitude of the velocity of nuclear strain as a function of time and for 3 different ECM stiffnesses. The velocity is greater in softer phenotypes as they possess less lamin-A,C and lamin-B than stiffer phenotypes like heart, for which the velocity is relatively lower. **(B)** Evolution of the magnitude of nuclear strain acceleration as a derivative of spreading velocity. It is clear that the velocity and acceleration are not linear, meaning that the spreading velocity will be affected by time. **(C)** Evolution of the magnitude of nuclear strain acceleration over a larger timescale, presented in log-log scale, showing a convergence towards the same value of the acceleration for all different phenotypes, independent on the stiffness. In (A) and (B) a timescale of less than 60s was selected as the stress fibers cycles are reported to range in the 10-30s, time periods much lower than the convergence time.