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Specific mechanical behaviours of healthy and cancer cells via a new model for cells and actin networks

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Abstract: Understanding the viscoelastic nature of the cells and their response to the mechanical stimuli provides the knowledge to analyse some behaviours of the cells. Although different elements of the cell are important for its mechanical properties but experimental data highlights actin cytoskeleton as the most important element. In this study, experimental data from previous studies including storage and loss moduli of different actin networks, networks with different cross-linkers or without them, and also creep behaviour of different cells are extracted to propose a new model. This newly presented model provides an insight to the viscoelastic properties of both actin networks and cells which are closely related to each other. It can be pointed out that the ability of this model to define the relations between concentration of actin or its cross-linkers and the adjustable parameters of the model, makes it a generic model. This model could be used to explain different mechanical behaviours of the healthy and cancer cells, including the decrease in the stiffness of the cancer cells, in terms of the changes in cross-linkers' concentration. From biomechanical view, changes in actin cross-linkers' concentration or type is one of the most important variations in cancer cells during their malignant transformation, which greatly affects their behaviours such as extracellular matrix detachment, deformability, and mobility. The knowledge of these dis-regulations of cancer tissue could be useful in their prediction, diagnosis, and treatment.

Keywords: Cell Mechanics; Mechanical Model; Actin Cytoskeleton; Viscoelasticity; Cancer Cell; Malignancy.

Introduction

The cytoskeleton as a biopolymer is a structure, which is responsible for cell's mechanical properties, determines the cell shape, and it is partially accountable for anchoring the cell to ECM or other cells. In some cells, it speeds up the material transport inside the cell [1]. Microfilaments (actin filaments), Intermediate filaments (IFs), and microtubules are three main protein filaments that consists the cytoskeleton. Since these filaments have different mechanical properties and dispersion throughout the cell, each one plays different part in the cell mechanics [2].

Actin as a semiflexible biopolymer [3] determines most of the mechanical behaviours of the cell such as viscoelasticity [4, 5] while based on the experimental studies mechanical behaviour of the actin networks and the cells are similar [6, 7]. Although this similarity is impressive, the absolute magnitudes for the storage modulus and the loss modulus, which indicate their dynamic mechanical properties, are different by 5 orders of magnitude [6]. There are some practical methods by which actin proteins are extracted from muscle and non-muscle cells and polymerized again in *in vitro* conditions the same as physiological conditions [8, 9]. In this way mechanical properties of actin networks independent of other cellular elements could be studied.

It is crucial for the cells to maintain their mechanical stability and structural integrity but at the mean time to be able to restructure and reorganize cytoskeletal networks with a suitable rate. This is mainly feasible by the presence of the actin networks and its related proteins. These proteins bind the actin filaments to each other or other elements inside and outside the cell. A large group of these proteins are actin cross-linkers, which determine most of the properties of the actin networks such as their microstructure, viscoelastic properties, and dynamics [10].

There are considerable numbers of models which describe the mechanical properties of the cells or actin networks. Walcott *et al.* has introduced a model which considers mechanics of actin networks based on the effect of myosin in stress fibre formation. This model has idealized the cytoskeleton as a randomly oriented number of rigid filaments in 2D and is used in sensing the elasticity of the

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substrate by the cell and more specifically its cytoskeleton [11]. Lieleg *et al.* has introduced their model for actin networks based on experimental data on cross-linked actin networks with Heavy Meromyosin (HMM) [12]. Some other studies have proposed that both cytoskeletal networks [13] and cells [14] exhibit power-law behaviour. It should be noticed that power-law behaviour of the storage modulus, which represents elasticity, for cells and cytoskeletal networks is controversial and exists only for frequencies in the range of 10^0-10^2 Hz [15] while different cells may experience loading frequencies different than this range.

Standard Linear Solid (SLS) (Figure 1A) is a model based on combination of dashpots and springs that describes some of the mechanical properties of the cells and different cytoskeletal networks. This model intends to describe a wider range of frequencies and has been used in both previous studies [16, 17] and even latest ones [18]. It has also been used for different semiflexible polymers [7]. It is used to describe the creep response of the cells such as leukocytes, neutrophils, and smooth muscle cells [16, 19, 20]. Although it is a general model that is developed many years ago, but its ability in describing cell mechanics is approved in recent studies [21, 22]. As the cells and actin networks are similar in mechanical behaviour and actin networks are considered to be semiflexible polymer, it is expected that SLS would be capable of explaining actin networks mechanics but our study shows that it has some shortages in describing the frequency behaviour of the actin networks. Since this model is one the most commonly applicable models of viscoelastic materials, overcoming these shortcomings would result in a more universal model which is also applicable in the field of cell biomechanics.

In order to characterize viscoelastic behaviour of different materials the Boltzman approach could be used [17, 23]:

$$\sigma(\mathrm{t}) = \int_{-\infty}^{t} G(\mathrm{t}-\tau)d\varepsilon(\mathrm{t}) \qquad \textbf{(1)}$$

Here $\sigma(\mathrm{t})$ and $\varepsilon(\mathrm{t})$ are stress and strain, respectively, that vary with time t, G(t) is the relaxation function and τ is the relaxation time. Considering the strain to be sinusoidal Equation 1 yields to:

$$\sigma(t) = \varepsilon_0 G'(\omega)\sin(\omega t) + \varepsilon_0 G''(\omega)\cos(\omega t) \qquad \textbf{(2)}$$

in which G'(ω) is storage modulus and G"(ω) is loss modulus, representative of the network's viscosity. Equations 3 and 4 represent the SLS model description for viscoelastic networks especially semiflexible polymers [7]:

$$G'(\omega) = \frac{[C_1^{\,2}(K_1+K_2)]\omega^2 + K_1K_2^{\,2}}{(C_1\omega)^2 + K_2^{\,2}} \qquad \textbf{(3)}$$

$$G''(\omega) = \frac{(C_1K_2^{\,2})\omega}{(C_1\omega)^2 + K_2^{\,2}} \qquad \textbf{(4)}$$

The parameters K_1, K_2, and C_1 are components of the model and ω is the angular velocity of the imposed loading. If the applied stress is in Pascal (N/m^2), the elastic components, K_1 and K_2, would have dimension of N/m^2 and viscose component, C_1, would have dimension of $N.s/(m^2)$. Equation 5 represents the description of the SLS model for creep behaviour of the cell.

$$\mathrm{d}(t) = \ell^{-1}\left[\frac{c_1 s + k_2}{(k_1c_1 + k_2c_1)s + (k_1k_2)}\mathrm{F}(s)\right] \qquad \textbf{(5)}$$

where F(s) is the Laplace transform of f(t), the applied mechanical force, and d(t) is the resultant deformation. The symbol ℓ^{-1} represents the mathematical operation inverse Laplace. If the applied load is in Newton (N), the elastic components, k_1 and k_2, would have dimensions of N/m and viscose component, c_1, would have dimension of N.s/(m).

The actin networks based on their frequency behaviour could be categorized to two categories. The first category includes networks without cross-linker [24] or with a cross-linker such as filamin [25]. In such networks, loss modulus begins from small amounts and monotonically increases with the frequency in intermediate amounts and tends to a final amount. The second category consists of networks with cross-linkers such as HMM or α-actinin [26, 27]. The loss modulus in this category increases with frequency from early small values but decreases suddenly in intermediate frequencies and then again increases to a final quantity. In both categories, the storage modulus, the same as other semiflexible polymers, increases with the frequency from the initial amount to the final amount [7]. Comparing the storage and loss modulus predicted by the SLS model with the experimental data shows that this model is capable of describing the storage modulus of both categories but its fit to the loss modulus is not good enough in the second category of the actin networks. In some cases in the second category, the loss modulus of the SLS model even diverges from the experimental data (negative R-square, a parameter that is used to evaluate the quality of the models in describing experimental data which is always less than or equal to the unity, and amounts closer to the unity show better fit). However, because of the similarities between mechanics of the cell and actin networks, any proposed model for the cell mechanics is expected to describe the behaviour of the actin network as well.

Despite these facts, it is possible to modify the SLS and propose a new model that describes all of the discussed mechanical properties of both cells and actin networks. The newly proposed model in this study (the Four-Element Model, FEM (Figure 1B))

is broadly in agreement with experimental data of mechanics of different actin networks as function of both frequency and time. In the next step the amount of models' parameters are related mathematically to the concentration of the actin or its cross-linkers. In this study experimental data from literature are extracted and SLS and FEM explanation of these data are compared to each other. The R-square is used in order to show the superiority of FEM over SLS.

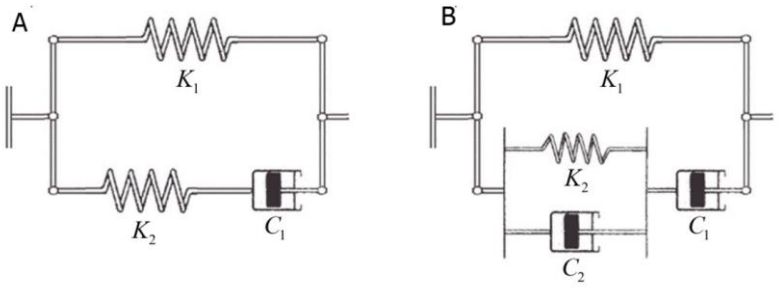


Figure 1. A) Standard Linear Solid Model (SLS) and B) the Four Element Model (FEM)

Methods

As described above, the SLS model is used for both the cells and the semiflexible polymers. Although actin networks are considered to be semiflexible, this model couldn't explain the frequency dependent mechanical properties of all types of these networks. The SLS model could be reclaimed to adopt the creep and the frequency dependent mechanical properties of the cells and the actin networks. In order to do this, it is necessary to find the relationship between components of the model and the network structure. In the SLS model the springs K_1 and K_2, represent the elasticity of the structure. K_2 reacts at the beginning of the loading where in the networks, entanglements (physical interactions of different strands) and cross-linkers (chemical linkage of strands) are largely influenced. Shortly after initiating the loading, the K_1 is affected and being stretched, when the actin filaments are acting. At all moments of the loading the component C_1 represents the viscosity of the network including the viscous behavior of entanglements, cross-linkers, and filaments [7].

Actin networks are formed in concentrations that are lower compared to other semiflexible polymers but their entanglement density is much higher than them [28]. This greatly increases the viscosity of the networks exactly in the initial moments of the loading where entanglements are highly effective. In other words, viscose effect of entanglements and cross-linkers is significant in comparison to their elasticity at the beginning of the loading. For this reason in order to improve the SLS model, a dashpot parallel to the K_2 spring, named C_2 is proposed.

Theoretical Modelling

In order to obtain the force-displacement relation for the FEM, the distribution of force and displacement for different elements of the model is used. This yields a differential equation which describes the overall relation between force and displacement of the whole model (Equation 6). This equation describes the time-dependent behaviour of the structure and includes force, displacement, and their first and/or second derivatives. The time dependent form of the model (Equation 8) that only includes force and displacement can be achieved using Laplace and inverse Laplace functions, respectively. Although stress and strain of viscoelastic materials are not in phase, but as the storage modulus of actin networks is much bigger than their loss modulus in almost all cases, they could be considered close to elastic materials and initial amounts of stress and strain to be zero.

$$(c_1+c_2)\frac{\partial \mathrm{f}(t)}{\partial t}+k_2\,\mathrm{f}(t)=c_1c_2\frac{\partial^2 \mathrm{d}(t)}{\partial t^2}+(k_1c_1+k_1c_2+k_2c_1)\frac{\partial d(t)}{\partial t}+k_1k_2\,\mathrm{d}(t) \quad \textbf{(6)}$$

$$\mathrm{F}(s)[(c_1+c_2)s+k_2]=D(s)[(c_1c_2)s^2+(k_1c_1+k_1c_2+k_2c_1)s+(k_1k_2)] \quad \textbf{(7)}$$

$$\mathrm{d}(t)=\ell^{-1}\left(\frac{(c_1+c_2)s+k_2}{(c_1c_2)s^2+(k_1c_1+k_1c_2+k_2c_1)s+(k_1k_2)}\mathrm{F}(s)\right) \quad \textbf{(8)}$$

Where F(s) and D(s) are the Laplace transform of f(t), the applied mechanical force, and d(t), the developed displacement, respectively.

For the creep response of the cell, force should be a step function. The creep response of the model is:

$$\mathrm{d}(t)=\ell^{-1}\left(\frac{(c_1+c_2)s+k_2}{(c_1c_2)s^2+(k_1c_1+k_1c_2+k_2c_1)s+(k_1k_2)}\left(\frac{1}{s}\right)\right) \quad \textbf{(9)}$$

Where $\frac{1}{s}$ is Laplace transform of unit step function.

The frequency behaviour of the actin networks is evaluated under sinusoidal force field. The storage and loss moduli of FEM could be calculated by solving Equation 2 for it:

$$G'(\omega)=\frac{[K_2C_1^{\,2}+K_1(C_1+C_2)^2]\omega^2+K_1(K_2)^2}{(C_1+C_2)\omega^2+(K_2)^2} \quad \textbf{(10)}$$

$$G''(\omega)=\frac{[C_1C_2(C_1+C_2)]\omega^3+(K_2^{\,2}C_1)\omega}{(C_1+C_2)^2\omega^2+(K_2)^2} \quad \textbf{(11)}$$

Experimental Data Explanation

Experimental data on creep test of different cells are extracted from literature and used to evaluate the ability of the Equation 9 of the FEM in describing time-dependent behavior of the cells in terms of R-square. The same is done for frequency dependent behavior of actin networks. Experimental data on actin networks without any kind of cross-linker and networks with three different cross-linkers are used. In each of these studies three different concentrations for actin or its cross-linker has been examined. The components of the FEM are evaluated to see whether they have meaningful relation with actin or cross-linkers concentration. In other words, it is necessary for the FEM components to vary reasonably with the concentration of actin or its cross-linker.

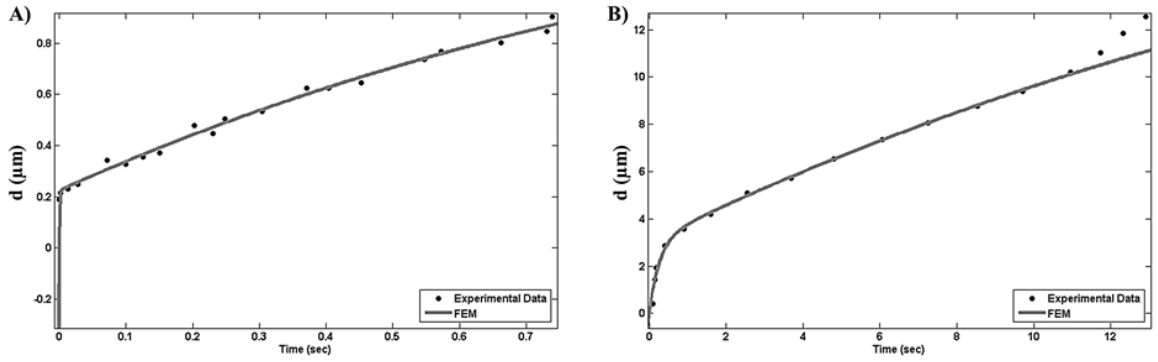
One of the most important features of actin networks that affect cellular behavior is the rapidity of their response to external loading. It is also important to know how much energy they damp in their structure in each cycle of loading. The calculated amounts for components of the model in each study are used to draw stress-strain diagram for each network. The area inside this diagram represents the damped energy in each cycle of loading. Equation 9 and related amounts of the components are also used to represent the rapidity of the response of these networks to external unit step loading. The final deformation of the network after a long time (peak value) and the time in which the deformation of the network reaches to the 95% of the final displacement (rise time) are used to compare behavior of different networks in response to the same loading condition.

Results

The FEM is used to describe creep behaviour of different cells. As discussed before the SLS model has described these cells perfectly but the FEM (Equation 9) shows a better fit (Table 1 and Figure 2) to these data [16, 19, 20].

Table 1. The SLS and FEM Explanation of Frequency Dependent Behaviour of Actin Networks

Cell Type	Model's Description of Experimental Data (R-square)		Reference
	SLS	FEM	
Leukocyte	0.9925	0.9928	Schmid *et al.* [16]
Neutrophil	0.9733	0.9944	Drury *et al.* [19]
Smooth muscle	0.9867	0.9873	Liao *et al.* [20]



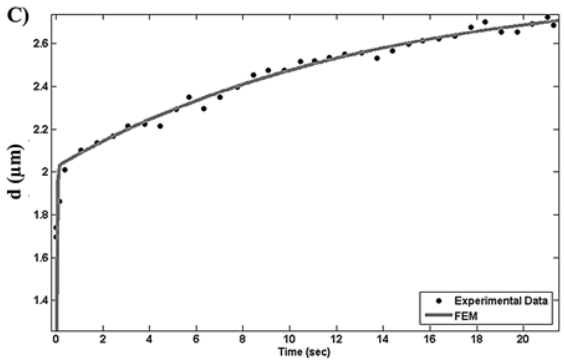


Figure 2. FEM EXPLANATION of Experimental Data in Creep Test of Different Cells: A) Schmid *et al.* Study [16], B) Drury *et al.* Study [19], and C) Liao *et al.* Study [20]

The ability of the FEM in explaining mechanics of actin networks is evaluated through comparing it with experimental data on actin networks without cross-linker or with filamin, which could be categorized in first category of actin networks and actin networks with HMM and α-actinin that are included in second category are also studied. As presented in Table 2, both models have equal ability in describing the storage modulus of these networks. Although SLS is good enough in describing loss modulus in the first category, FEM describe these data even better. It is also important to notice that the SLS explanation is not good enough in most cases of second category and it is often divergent from experimental data. The improvement of the FEM compared to SLS (presented as the percent of increase in R-square) is significant.

Table 2. Comparison between the SLS and FEM Explanation of Frequency Dependent Behavior of Actin Networks in Four Different Studies

Actin Concentration ($mg.mL^{-1}$)	**Cross-linker's Relative Concentration**	**Model's Description of Experimental Data (R-square)**			**Improvement in Conformity of the Model (%)**
		G' (SLS and FEM)	**G" (SLS)**	**G" (FEM)**	
Actin solution; Schmidt *et al.* [24]					
0.5	0	0.9766	0.8749	0.9692	9.43
1		0.9855	0.8242	0.9573	13.31
2		0.7445	0.6912	0.9519	26.07
Actin networks with HMM; Luan *et al.* [26]					
0.4	0.0013	0.9294	-0.9042	0.7335	163.77
	0.01	0.9606	-2.198	0.5371	273.51
	0.02	0.9388	0.3212	0.6669	35.47
Actin networks with filamin; Schmoller *et al.* [29]					
0.4	0.005	0.9452	0.8774	0.9678	9.04
	0.01	0.9352	0.8039	0.9378	13.39
	0.02	0.943	0.754	0.9092	15.52
Actin networks with α-actinin; Ward *et al.* [27]					
1	0.005	0.9519	-2.711	0.2811	299.21
	0.01	0.9598	0.5358	0.8521	31.63
	0.02	0.9443	0.274	0.7138	43.98

Parameters in the SLS does not vary with concentration of actin (C mg/mL) or cross-linker relative concentration ($R=C_{cross\text{-}linker}/C_{actin}$) in a way that can be described mathematically. Not only the FEM's description of all actin networks is admissible, it is important to define a reasonable relation between amounts of parameters for networks with different concentrations of actin or its cross-linkers. As discussed earlier, components K_1 and C_1 represent elasticity and viscosity of the filaments respectively and are chosen from the fit of storage modulus to experimental data. In all four different studies that are examined, both K_1 and C_1 increase linearly (Y=aX+b) with C or R. Components K_2 and C_2 that are responsible for elasticity and viscosity of entanglements and cross-linkers in the actin networks, respectively, and are extracted from the model's explanation of the loss modulus data. In the networks of first category both of them decrease linearly (Y=aX+b) with C or R while in the second category both of them decrease as a rotational function (Y=a/(X+b)) with R (Table 3 and Figure 3).

Table 3. Variation of FEM Components with Actin Concentration or Cross-Linker Relative Concentration

Actin Concentration (mg/mL)	**Crosslinker's Relative Concentration**	**Components of the Model**			
		K_1	**K_2**	**C_1**	**C_2**
Actin solution; Schmidt *et al.* [24]					
0.5	0	0.1138	1.608	0.7137	0.1656
1		0.2235	1.245	1.403	0.1477
2		0.3082	0.4388	3.447	0.115
Type of fit		linear	linear	linear	linear
R-square		0.9318	0.9994	0.9243	0.9995
Actin networks with HMM; Luan *et al.* [26]					
0.4	0.0013	0.409	0.4579	4.249	0.03487
	0.01	0.9067	0.1651	12.66	0.007942
	0.02	3.853	0.1635	51.85	0.002654
Type of fit		linear	rotational	linear	rotational
R-square		0.8828	0.9482	0.9027	0.997
Actin networks with filamin; Schmoller *et al.* [29]					
0.4	0.005	0.264	7.629	0.2906	0.1545
	0.01	0.5724	5.902	0.5066	0.1246
	0.02	0.765	2.406	0.5587	0.1148
Type of fit		linear	linear	linear	linear
R-square		0.8994	1	0.7449	0.7912
Actin networks with α-actinin; Ward *et al.* [27]					
1	0.005	19.25	1.45	37.67	0.006882
	0.01	60.97	1.118	121	0.002824
	0.02	95.13	0.7655	202.6	0.002524
Type of fit		linear	rotational	linear	rotational
R-square		0.9399	1	0.962	0.9036

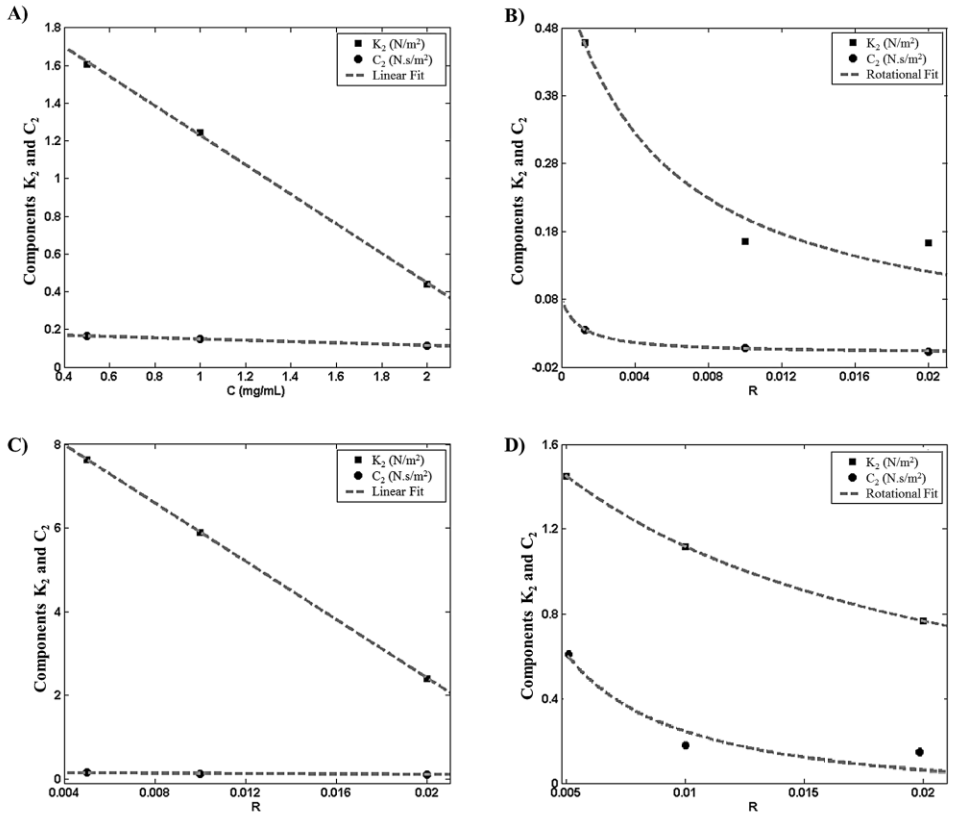


Figure 3. Components of the FEM, K_2 and C_2 Respectively for A) Schmidt *et al.* [24], B) Luan *et al.* [26], C) Schmoller *et al.* [29], and D) Ward *et al.* [27] Studies

The other aspect of actin networks that is reviewed is their response to unit step loading. All of the discussed networks show less deformation in response to the same external loading, as the concentration of actin or its cross-linker increases, but the response becomes more rapid in networks with cross-linker as its concentration increases and networks without cross-linker respond more slowly as the actin concentration increases (Table 4 and Figure 4).

Table 4. Response of Different Actin Networks to Unit Step Loading (Creep Test)

Actin Concentration (mg/mL)	**Crosslinker's Relative Concentration**	**Response to unit step loading**	
		Rise Time	**Peak**
Actin solutions; Schmidt *et al.* [24]			
0.5	0	19.5805	8.7855
1		20.9381	4.4723
2		46.9384	3.2436
Actin networks with HMM; Luan *et al.* [26]			
0.4	0.0013	46.3746	2.4425
	0.01	101.9526	1.1022
	0.02	0.1643	0.2515
Actin networks with filamin; Schmoller *et al.* [29]			
0.4	0.005	3.3447	3.7865
	0.01	2.8091	1.7438
	0.02	2.6189	1.3056
Actin networks with α-actinin; Ward *et al.* [27]			
1	0.005	9.4109	0.0519
	0.01	0.0525	0.0162
	0.02	8.19E-05	0.0104

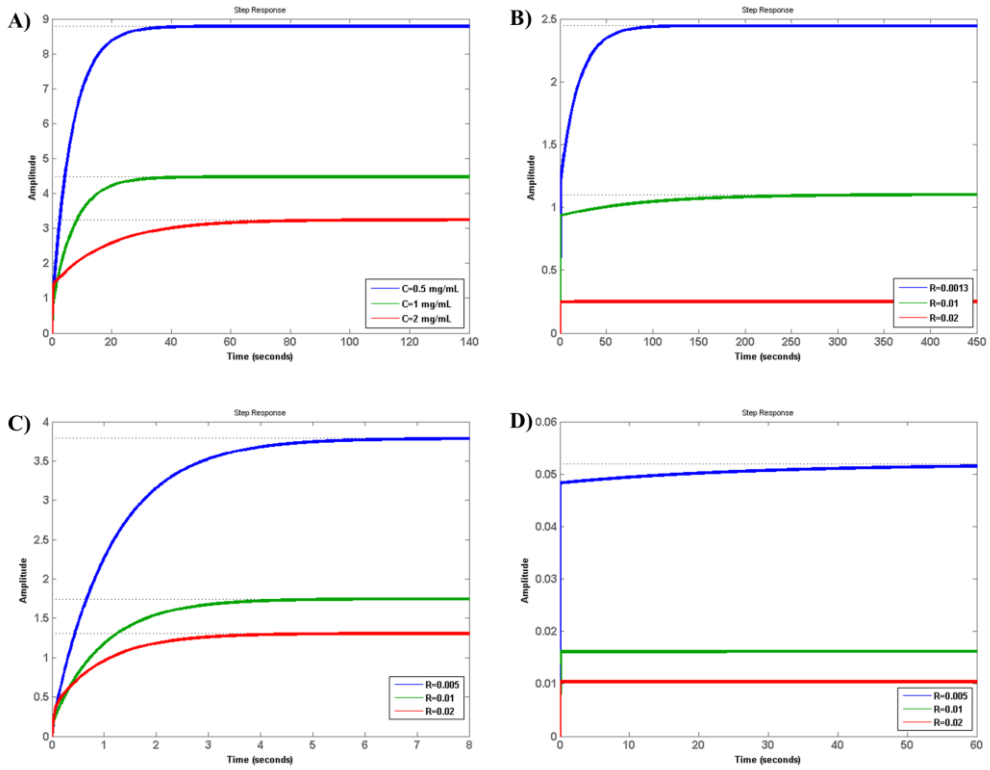


Figure 4. Response of Different Actin Networks to Unit Step Loading (Creep Test), A. Without Cross-linker, B. HMM as Cross-linker, C. Filamin as Cross-linker, D. α-actinin as Cross-linker

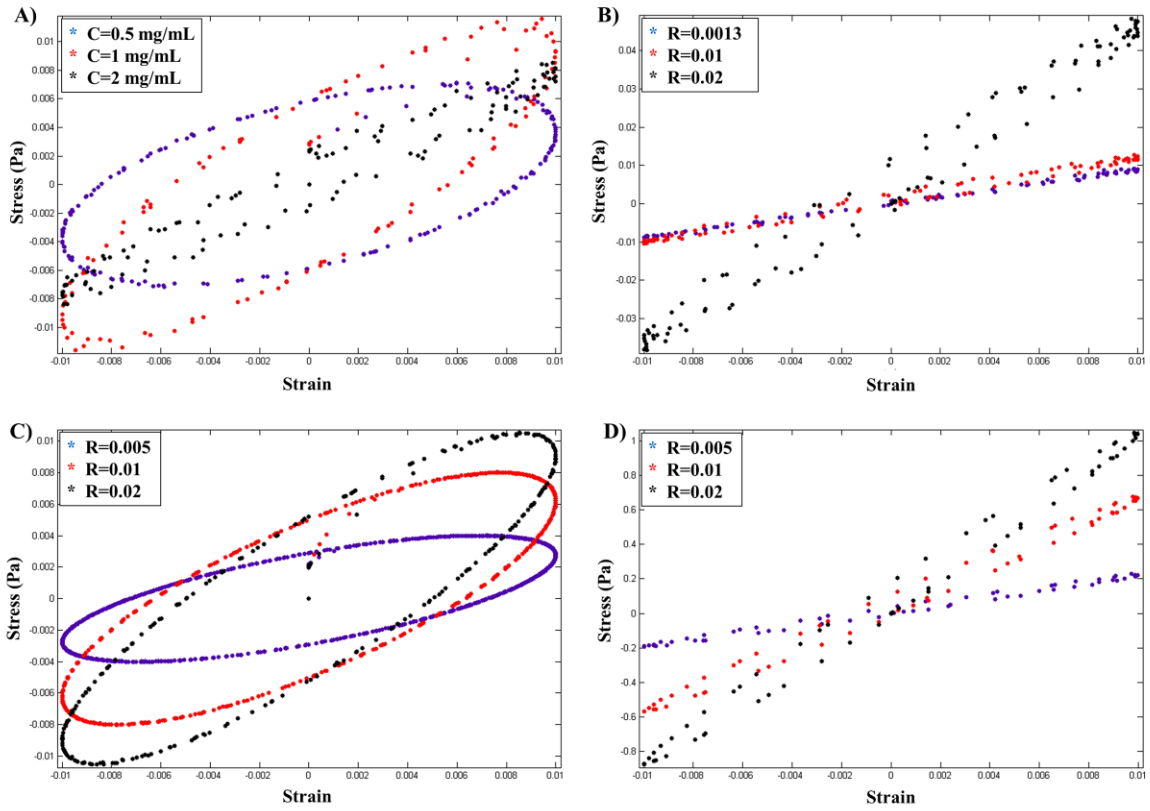


Figure 5. Lissajoux Diagram for Different Actin Networks, A. Without Cross-linker, B. HMM as Cross-linker, C. Filamin as Cross-linker, D. α-actinin as Cross-linker

Lissajoux is the name that is used for stress versus strain diagrams in which the area inside the diagram represents the amount of dissipated energy inside a network. Figure 5 represents the lissajoux diagram for all four different studies. As it is obvious, in the networks without cross-linker, there is no definite relation between actin concentration and the damped energy (Figure 5, A) but in networks with cross-linker, independent of the cross-linker's type, the damped energy increases with the relative concentration of the cross-linker (Figure 5, B-D).

Discussion

One of the most important usages of the SLS model is to describe mechanics of different cells [5, 16, 17], and as discussed it is suitable in this field. The FEM which is supposed to be an alternative for the SLS is also perfect in this field of cell mechanics and describes creep behavior of the cells even better than the SLS (R-squares closer to 1 in Table 1).

One of the other applications of the SLS model is describing different mechanical properties of semiflexible polymers. Since actin networks with different cross-linkers are considered to be semiflexible, and also because of the similarities between mechanical behavior of the cells and the actin networks, the SLS is expected to be capable of explaining actin networks. The same prospect exists for the FEM. But as discussed, this is not a reasonable expectation for the SLS. As it is shown in Table 2, the FEM not only shows a great improvement in describing different actin networks (an average of 77.86% improvement in describing loss modulus of the 12 different actin networks) over the SLS but also its description in almost all cases is completely acceptable. It should be noticed that the general form of storage modulus for both models is the same and they both have similar and appropriate fit to experimental data (Table 2). Figure 6 is an example of superiority of FEM over SLS in explaining loss modulus data.

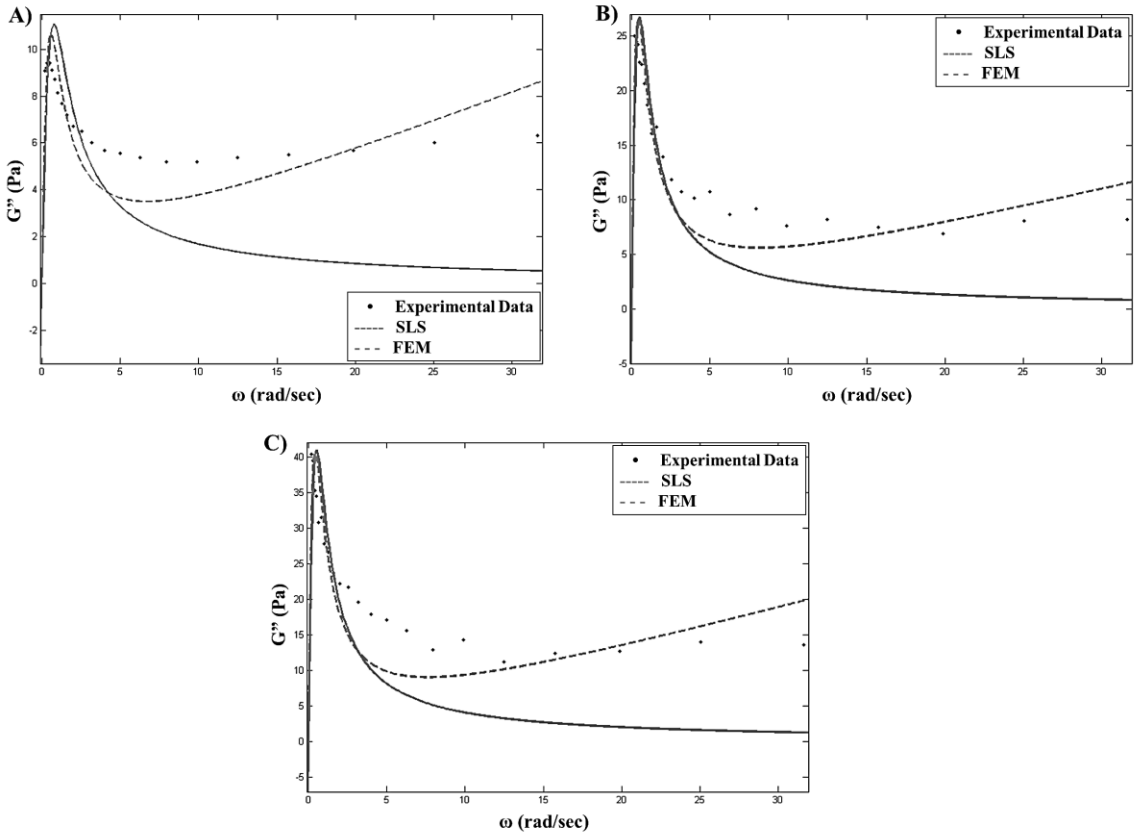


Figure 6. Comparison Between SLS and FEM in Explaining Experimental Data on Storage Modulus of Actin Networks in Ward *et al.* [27] Study, A) R=0.005, B) R=0.01, and C) R=0.02

Adding a number of springs or dashpots to the SLS model in any way, as will increase its dependency on the frequency from a first-order to a second-order polynomial, will provide a better fit to the experimental data, but the point is that adding a component blindly will cause the components' amount to vary irregularly with actin concentration or cross-linker relative concentration in each case while in FEM all of the components have a mathematical relationship with C or R (Figure 3).

Although it is not possible for FEM components' to attribute to cellular and more precisely cytoskeletal structure, the way components vary with the concentration could be related to the structure of the network indirectly. Experimental studies show that in networks with cross-linkers such as HMM and α-actinin, in intermediate frequencies, thermal unbinding of the cross-linker causes a sudden decrease in loss modulus [10] so any network with unknown cross-linker in which both K_2 and C_2 decrease with R, in all likelihood would have experience cross-linker unbinding in intermediate frequencies. As discussed, in the FEM the components K_2 and C_2 are postulated to be responsible for elasticity and viscosity of the entanglements and cross-linkers, respectively. In such networks which experience cross-linker unbinding, both the K_2, and C_2 decrease more rapidly compared to the same components of the networks that don't experience this kind of unbinding. This shows the substantial effect of entanglements and cross-linkers in these networks.

One of the facts about both the SLS and FEM is their disability in describing storage modulus of all networks in small frequencies, mostly frequencies less than 1 Hz (Figure 7). This is a common problem for all models since actin networks behavior in this range of frequencies is not frequency-dependent [30].

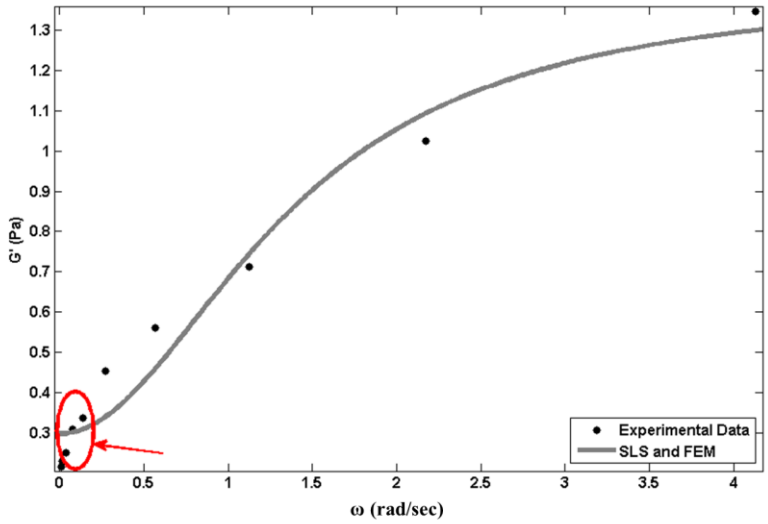


Figure 7. Storage Modulus of Both SLS and FEM in Small Frequencies

Based on these facts the FEM covers the discussed weaknesses of the SLS and describes mechanics of both cells and actin networks. This makes the FEM a suitable model for justification of mechanical behavior of cancer cells in which the protein structure of actin and its cross-linkers remains constant while the concentration and type of the actin cross-linkers alters [31]. HMM (140 kDa) is one of the actin cross-linkers that is mostly present in muscle cells [32]. Studies on different cancer cells such as basal cell carcinoma, squamous cell carcinoma, and mammary carcinoma cells reveals that although HMM is not present in the healthy state of these cells, in the cancer state of them there is an increased amount of this protein. These studies show that this increase is related to the malignant growth of the cancer cells and their invasion [33]. Filamin (280 kDa) is a non-muscle protein that acts as actin cross-linker [32]. Different studies has proven that presence of filamin is important in breast and prostate cancer, exactly in the metastasis of these cancer cells [31]. Based on these studies, preventing this protein's expression in cancer cells reduces their mobility and re-expression of it restores that [34, 35]. α-actinin is another actin cross-linker (103 kDa) [32] where its presence is important in breast, ovary, pancreas, lung, and astrocytoma cancers [31]. Based on recent studies expression of α-actinin in cancer cells enhances their motility [36].

Our findings are consistent with these experimental studies. As discussed, one of the most important results of the increase in cross-linkers concentration is increase in the structure's mobility. As it is obvious in Table and Figure 4, as the concentration of all three cross-linkers increases, the networks show less but rapid deformation which is consistent with what is happening in cancer cells in their invasion and metastasis. As it is obvious in Figure 5, the increase in all three cross-linkers concentration enables the cancer cells to damp more energy and in this way they would be more susceptible to external loadings and environmental conditions.

Increase in actin concentration causes the network and consequently the whole cell to act more slowly (Table 4). It is also obvious from Figure 5 that there is no logical relationship between damping ability of the actin networks and its concentration. Based on these results, increase in actin concentration of cancer cells, unlike cross-linkers, won't provide appropriate conditions for their invasion and metastasis.

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