Totla: 8.5

Some errors are not analysed.

Computational Mechanics

Course Work: Elastic Sensors

Supervisor: Jose Muñoz

Students: [redacted], [redacted] and [redacted]

May 2019

Table of contents

1 Introduction

Increasing evidence suggests that mechanical cues might play a critical role in the proliferation and development of cellular organisms. In particular studies have shown how these alone can trigger malignant phenotype in otherwise non-malignant cells. Despite their importance, mechanical signal these are too often overlooked due to the fact that it remains remarkably difficult to collect accurate extensive measurements.

The article “Cell-like pressure sensors reveal increase of mechanical stress toward the core of multicellular spheroids under compression” focuses on developing a novel method for reliably measuring how pressure propagates inside the tumor. The method presented in the article was developed and tested on multi-cellular spheroids (MSC) under isotropic compression. MSC are spherical aggregates of cell that are commonly used to mimic the behaviour of tumor cells, while making experiments considerably more convenient and reproducible in a lab environment. Besides describing the method by which pressure is measured, the article describes how stress propagates inside the tumor cells in a non-trivial manner, and underlines its relations with cells proliferation and development. The main finding is that the mechanical stress increases toward the core of MSC, and we will further elaborate on the causes and effects this phenomena has in Section 6.

The method developed in the article relies on computing the stress in the MSC from the measured volumetric strain of small cell-like micro-sensors that have been inserted in the MSC (referred to as microbeads). The bulk-modulus of the microbeads is derived experimentally by controlling the pressure and measuring the volumetric strain of the microbeads, and its calculation allows the stress to be directly computed form the volumetric strain.

2 Advantages of PAA microbeads

The sensors used to measure the volumetric stress are polyacrylamide gel microbeads. Polyacrylamide (PAA) is a polymer formed from acrylamide sub-units. Polyacrylamide gels use is quite widespread when it comes to studying cells behaviours, since they present several advantageous properties such as:

1. Compressibility
2. Homogeneous elastic properties
3. Elastic modulus can be tuned by controlling the concentration of its components (acrylamide to bisacrylamide)
4. Biocompatibility
5. Inertness

6. They promote cellular adhesion
7. They can be loaded with fluorophore to facilitate imaging
8. Cells and their protrusions do not enter the gel due to the small pore exclusion size of PAA gels

3 The bulk modulus

The bulk modulus (expressed with the Greek letter κ) measures how resistant a certain material is to compression. It is mostly used to study the behaviour of fluids, although it applies to any substance. It can be used to predict compression, calculate density, and indirectly reflect the type of chemical bonding within a substance. It depends on the state of the matter (that is solid, liquid or gas) and, in some cases, on the temperature of the material.

For example, if we have a substance with a small bulk modulus, it means that it has a high compressibility. The bulk modulus is the ratio between the pressure exerted on the material and the volumetric strain caused by it. It is also measured in Pascals [Pa = N/m^2], since the volumetric strain is a unitless quantity.

For example, the bulk modulus for air is $\kappa = 101$ kPa, which means that by increasing the applied pressure to 1,01 kPa, its volume will be reduced by 1%. In the case of water, the bulk modulus is much larger, $\kappa = 2,15$ GPa, since it is much more difficult to compress. Solids, such as diamond, with $\kappa = 443$ GPa, have usually an even higher bulk modulus.

If a material behaves linearlly, knowing the value of the bulk modulus together with Young modulus and shear modulus allows us to predict the material's response to stress or strain.

3.1 Derivation of bulk modulus

The bulk modulus can be expressed as the ratio between the pressure P and the volumetric strain $e = tr(\boldsymbol{\varepsilon})$.

$$\kappa = V\frac{\Delta P}{\Delta V}$$

where V is the initial volume and ΔV is the change in volume due to ΔP. We first derive the expression for volumetric strain. We know that:

$$e = tr(\boldsymbol{\varepsilon}) = \frac{\Delta V}{V}$$

.

From the constitutive equations we have:

$$\boldsymbol{\varepsilon} = -\frac{\nu}{E} tr(\boldsymbol{\sigma})\mathbf{1} + \frac{1+\nu}{E}\boldsymbol{\sigma}.$$

If we substitute that in the expression for volumetric strain, it yields:

$$e = tr(\boldsymbol{\varepsilon}) = -\frac{\nu}{E} tr(\boldsymbol{\sigma}) tr(\mathbf{1}) + \frac{1+\nu}{E} tr(\boldsymbol{\sigma}) = \frac{1-2\nu}{E} tr(\boldsymbol{\sigma}).$$

Now, as we are in hydrostatic pressure, we can write the trace of $\boldsymbol{\sigma}$ as $3\sigma_m$. Therefore,

$$e = \frac{3(1-2\nu)}{E}\sigma_m$$

From this last equation we can drive the final expression for the bulk modulus, as it can be expressed as the ratio of the pressure and the volumetric strain:

$$\kappa = \frac{\sigma_m}{e} = \frac{E}{3(1-2\nu)}$$

3.2 Verification of the previous formula

It is possible to verify the previous formula with a simple experiment: applying an hydrostatic pressure to a discretised square domain using the finite element method, and measuring the volume changes versus the applied pressure.

To do so, a MATLAB script has been used in order to implement the finite element method to solve the problem (*FemLab*). We used a square domain, under the hypothesis of plain strain, that is, $\varepsilon_{zz} = 0$ and $\sigma_{zz} = \nu(\sigma_{xx} + \sigma_{yy})$. Applying a hydrostatic pressure to the boundaries of the square domain, considering that $\sigma_{xx} = \sigma_{yy}$, the final volume is messured numerically to get the values of ε_{xx} and ε_{yy}.

Finally, to compute the resulting bulk modulus, the following formula has been used:

$$\kappa = \frac{\sigma_m}{e} = \frac{1}{3} \cdot \frac{tr(\boldsymbol{\sigma})}{tr(\boldsymbol{\varepsilon})}$$

which is equal to the expression

$$\kappa = \frac{E}{3(1-2\nu)}$$

with an error of $1,39 \cdot 10^{-4}$.

Why is there an error if solution should be linear? Have you checked error of linear system of equations? How many elements

3.3 Deduction of κ from experimental data

As mentioned in the previous sections, the bulk modulus is defined as the change in volume over a change in stress.

Once the bulk modulus of the microbeads has been computed (by controlling the osmotic stress and measuring the volumetric strain, as described in the article) knowing the stress-strain curve (Figure 1) of the microbeads allows to use them as elastic sensors.

We note that the bulk modulus corresponds to the slope of the curve at the origin point in Figure 1 , as it represents the ratio between the stress and the strain.

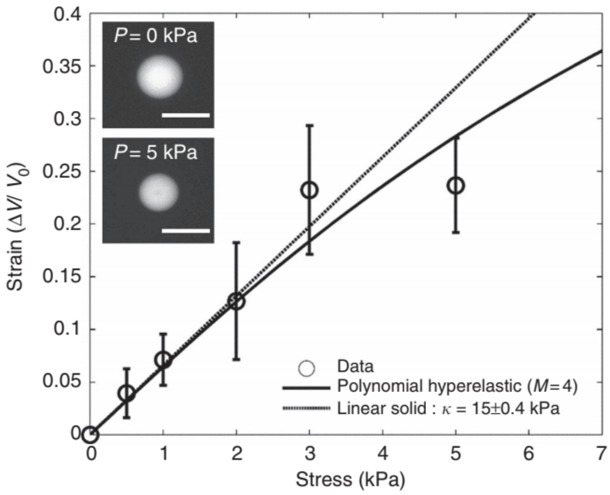

Figure 1: Determination of bulk modulus of PAA microbeads (5/0.225)

From the plot in Figure 1 we can read off some values to compute the bulk modulus as follows:

$$\kappa \approx \frac{5}{0,335} = 14,925\ kPa$$

which is within the values of the bulk modulus according to Figure 1 ($\kappa = 15 \pm 0.4$ kPa).

We take the slope at the **origin** because the experimental data in Figure 1 can be approximated linearly when the deformation is less than 15%. For larger deformations, the microbeads start plastifying, losing their elastic behaviour. In the plastic region, the sensors are no longer accurate in measuring pressure inside the cancer cells. ok

Finally, from observing the stress-strain curve, the sensors clearly exhibit a **hardening** behaviour, since we can observe that to an increasingly higher stress value corresponds a progressively small increase in the volumetric strain. Yes

4 The Rivlin-Moonly model

Normally when we can apply the constitutive equations, under the assumption that the displacements of a body are much smaller than any of the dimensions of that body (small deformation hypothesis). This allows us to reduce complex non-linear behaviour to a linearized form. However, sometimes we cannot avoid dealing with large strains, which makes the standard linearized approach inadequate. One of the most popular methods used to model large strains non-linear behaviours is the Rivlin-Moonly model. Unlike other ones it is important to notice that the Rivlin-Moonly model does not explain the intrinsic mechanical behaviour of the material and it is simply a polynomial curve-fit on the collected data.

One of the main insights about the Rivlin-Moonly model is that it first establishes a relationship between the strain energy W and the deformation of the material. This relationship is represented by the strain energy function which relates the strain energy density of a material to its deformation gradient.

$$W = \hat{W}(\mathbf{C}) = \hat{W}(\mathbf{F}^T\mathbf{F}).$$

From the strain energy density function it is possible to express the stress $\boldsymbol{\sigma}$.

$$\boldsymbol{\sigma} = \frac{1}{J}\frac{W}{\partial \mathbf{F}} \cdot \mathbf{F}^T$$

where $J := det(\mathbf{F})$.

We are now left with the problem of finding a polynomial expression for the strain energy density function such that it can be fitted to the data. To achieve this goal, some conditions have to be fulfilled:

1. The strain energy has to be written as a function of the principal strains
$$U = f(\lambda_1, \lambda_2, \lambda_3).$$
Or any other invariant....
2. The strain energy cannot depend on the orientation of the coordinate system, hence it must be a function of the invariants of $\mathbf{B} = \mathbf{F}^T\mathbf{F}$. C=F^T*F
3. $U = 0$ when $\lambda_1 = \lambda_2 = \lambda_3 = 1$ (i.e. when the strain is zero).
4. $J = 1$ when the material is incompressible.

Based on these conditions, a polynomial with arbitrarily high degree can be constructed in the form

$$W = \sum_i \sum_j C_{ij}(\bar{I}_1 - 3)^i(\bar{I}_2 - 3)^j + \sum_{k=1}^{m} D_k(J-1)^{2k}$$

where

$$\bar{I}_1 = J^{-2/3}I_1; I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2; J = det(\mathbf{F})$$
$$\bar{I}_2 = J^{-4/3}I_2; I_2 = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_3^2\lambda_1^2.$$

The constants, C_{ij} and D_k are computed by fitting the polynomial to the data. The number of terms of the expansion (and with it the degree of the polynomial) is determined by the data we are trying to fit (i.e. a too high degree will cause over-fitting of the model, while a low degree polynomial is likely too under-fit).

It is important to notice that since:

$$J = det(\mathbf{F}) = \lambda_1\lambda_2\lambda_3 = V$$

J=dV/dV0 approx (V-V0)/V0

we can conclude that:

$$J - 1 = e$$

where e is the volumetric strain.

The strain energy density is defined as:

$$U(\boldsymbol{\varepsilon}) = \int_a^b \boldsymbol{\sigma} : d\varepsilon$$

and the stress as:

$$\sigma_{ij} = \frac{\partial U(\varepsilon)}{\partial \varepsilon_{ij}}.$$

for small strains

If the stress $\boldsymbol{\sigma}$ is given and we know the expression of $U(\boldsymbol{\varepsilon})$, the corresponding values for the strain $\boldsymbol{\varepsilon}$ must minimize $U(\boldsymbol{\varepsilon})$. Only in the case of the Moonly-Rivlin model we are not after the values of $\boldsymbol{\varepsilon}$ or $\boldsymbol{\sigma}$ but rather after the parameters of a polynomial model that satisfies these characteristics.

We are interested in looking at the last term in the polynomial hyperelastic model

$$\sum_{k=1}^{m} D_k(J-1)^{2k}$$

as it appears explicitly in Figure 1. If we think in terms of minimization of $U(\boldsymbol{\varepsilon})$, the higher values for m and D_k we consider, the more we penalize higher values of $J - 1$ (volumetric strain) for a given $\boldsymbol{\sigma}$. This gives rise to a behaviour of the strain-stress curve that is very similar to hardening (it differs in the sense that in the Moonly-Rivlin model this does not arise form insights over the underlying mechanics, but artificially from the manipulation of the polynomial terms). Indeed this is consistent with the behavior we can observe in Figure 1.

5 Anisotropic behaviour and arching effect

To analyze the effect of anisotropic response of the cells to the stress experienced by the beads, a model was put forward in the article of Dolega et al. The model is used to compute the radial stress profiles of an anisotropic elastic sphere undergoing an isostatic compression. The radial stress profiles are computed in each of these three cases:

a) The cells are softer in the radial direction.

b) The cells are mechanically isotropic.

c) The cells are stiffer in the radial direction.

The effects on the radial stress profiles in each of these cases is reported in the following chart.

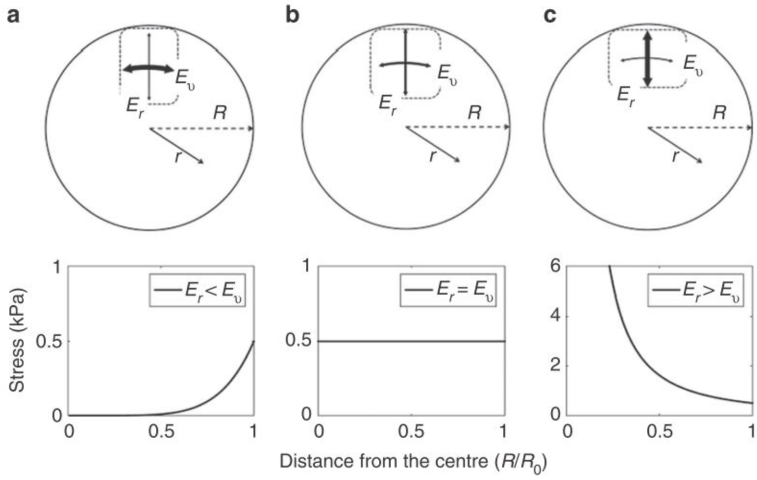

Figure 2: Stress distribution in dependence of the cellular anisotropy - Theoretical model.

When the cells are softer in the radial direction (case **a**) the outer layers are the ones experiencing more stress, and due to the "arching effect" progressively shield the inner layers of cells. This causes the radial stress to diminish for cells that are closer to the center of the spheroid. The situation is reversed in case **c**, with the stress increasing as we get closer to the center.

In the considered case the anisotropy is not constant throughout the spheroid, in fact it exhibits a transition from a region of high anisotropy to a region of lower anisotropy near the center.

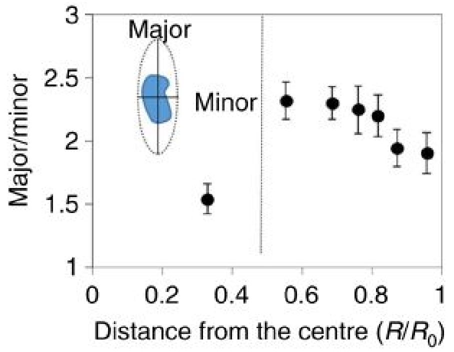

Figure 3: Organization of cells within CT26 spheroids (ratio of the major to the minor axis of the cell).

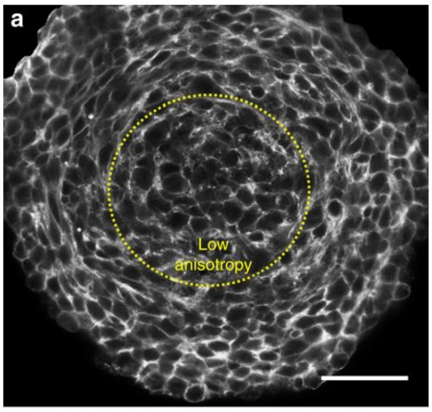

Figure 4: Organization of cells within CT26 spheroids (equatorial plane).

6 Conclusions

Growth arrest and proliferation have been proven to be triggered also by mechanical clues besides biochemical ones, and PAA gel microbeads play an important role in doing so. Yes

The article concludes that mechanical stress increases towards the center of the spheroid due to the cell anisotropy. Consistent with this conclusion, it can be observed that there is a drop in the stress near the core (Figure 5). This can also be due to the decrease in the anisotropy behaviour in the area immediately surrounding the center (Figure 3 and 4).

In the experiments, it has been observed that near the surface the cells continue to proliferate and actively rearrange them-self in such a manner to dissipate the compressive stress. As we get closer to the center, however, both rearrangement and proliferation behaviours are hindered by the increased jamming of the cells.

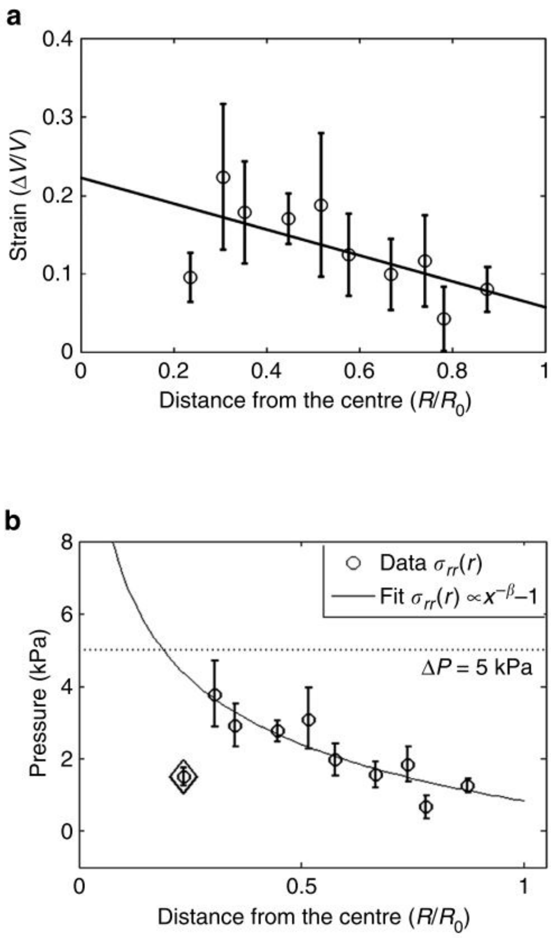

Figure 5: Pressure distribution in CT26 spheroids upon 5 kPa compressive stress.

7 References

1. M.E. Dolega, M. Delarue, F. Ingremeau, J. Prost, A. Delon and G. Cappello. Cell-like pressure sensors reveal increase of mechanical stress towards the core of multicellular spheroids under compression. Nature Communications, 8:14056, 2017

2. Article: Hyperelastic Mooney-Rivlin Model: Determination and Physical Interpretation of Material Constants. MIT International Journal of Mechanical Engineering, Vol. 6, No. 1, January 2016, pp. 43-46.

3. Article: A NONLINEAR VISCOELASTIC MOONEY-RIVLIN THIN WALL MODEL FOR UNSTEADY FLOW IN STENOTIC ARTERIES by Xuewen Chen.

4. Book: Continuum Mechanics for Engineers by X. Oliver and C. Agelet de Saracibar, September 2016, pp. 271-273.

Date?
Journal?

5. Website: *Wikipedia: Bulk modulus* [Accessed: 19/04/2019]

6. Website: *ThoughtCo: What Is Bulk modulus* [Accessed: 21/04/2019]