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## Mechanical Stimulation in Tissue Engineering

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**John Rasmussen, Cristian Pablo Pennisi, Tea Rasmussen, Marcelo Ferreira, Christian Gammelgaard Olesen and Vladimir Zachar**

Aalborg University, Denmark

Corresponding author: John Rasmussen <jr@m-tech.aau.dk>

### 5.1 Background and Introduction

It is well accepted that mechanical stimulation influences cell differentiation and growth. In the related field of pressure ulcers, investigations have revealed that mechanical deformations can lead to tissue necrosis. For a comprehensive review of theories explaining tissue necrosis under mechanical loads, please refer to Olesen, de Zee, & Rasmussen (2010) [1]. In other words, mechanics plays an important role for the entire lifecycle of cells and tissue in general. Methods for the consistent investigation of the related phenomena are therefore important.

This chapter reports on development of methods to impart controlled states of deformation to tissue samples *in vitro*. These methods are generally applicable for tissue growth but we shall focus on experimental investigation of cell necrosis in response to different loading conditions. This can possibly contribute to development of an injury criterion for tissue, which can have direct impact on healthcare practices.

#### 5.1.1 Mechanical Theories of Material Damage

Mechanics of materials is a well-developed area considering both macroscopic and microscopic behavior of materials. In this field, the concept of strength refers to the ability of a material or a structure to withstand loads without breaking or collapsing. The applied loads may be static, such as the self-weight of a bridge, or dynamic, such as the vibrations affecting the wing of an aircraft in turbulent air or the cyclic bending and torsion of a bicycle

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crankshaft. Common to these cases is the idea that the material is inert, i.e. does not change its properties except from the damage caused by the applied loads.

Stress and strain are central concepts in strength of materials, and the concept of strength is coupled to an idea of the local stresses or strains causing immediate or cumulative damage at each material point. For materials with linear behavior (which is usually the case for sufficiently small deformations) stresses are coupled to strains through Hooke's law, stating that

$$\boldsymbol{\sigma} = \mathbf{E}\boldsymbol{\varepsilon} \quad (5.1)$$

where  $\boldsymbol{\sigma}$  is the stress tensor,  $\boldsymbol{\varepsilon}$  is the strain tensor, and  $\mathbf{E}$  is the elasticity tensor. Two points are important for later application of this idea to living tissues. Firstly, stress cannot be measured but only calculated, whereas strain is derived directly from deformation, which can be experimentally observed. It may not be immediately obvious to the casual student of mechanics that force (and stress) are imaginary physical quantities, but it becomes obvious if we look at methods for measuring force: they are all based on some form of observation of deformation.

Secondly, all terms of Equation (5.1) are tensors, i.e. multidimensional properties. In its most general form,  $\mathbf{E}$  contains 81 components, which by means of symmetry conditions and thermodynamic considerations can be drastically reduced. All properties, however, remain multidimensional to some extent depending on the properties of the material in question. It therefore rarely makes sense to discuss "the strain" or "the stress" as though the property is one-dimensional.

It is therefore also impossible, except in much idealized cases, to appoint a single stress or strain level that breaks a given material. However, many technically important materials can with good approximation be assumed ductile, homogeneous and isotropic and, for such materials, the yield criterion of von Mises is well-established. This criterion is based on a scalar combination of components of the stress tensor. We shall call this scalar combination the von Mises stress. For a certain class of materials, it is well accepted that yield, i.e. permanent deformation, occurs when the von Mises stress exceeds a magnitude that is characteristic for the given material. It is important to notice that many different combinations of stress components can result in the same value of the von Mises stress and therefore lead to yield. Similar yield or failure criteria have been developed with varying success for more complex materials, for instance the Tsai-Hill [2] and Tsai-Wu [3] criteria for

composites or statistical failure prediction based on Weibull statistics [4] for ceramics.

Let us review the definition of the von Mises stress. A material has six stress components in its tensor. These components depend on the coordinate system, so the stress tensor changes when the reference frame is rotated, although the material remains in the same state. In other words, the same stress state in the same material point can be expressed by many different stress tensors. In its general form, a stress tensor contains the following components:

$$\boldsymbol{\sigma} = \begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{xy} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{xz} & \sigma_{yz} & \sigma_{zz} \end{bmatrix} \quad (5.2)$$

where indices  $x$ ,  $y$  and  $z$  refer to directions in the chosen coordinate system. The diagonal elements,  $\sigma_{ii}$ , are normal stresses and designate pure compression or tension of the material, and the off-diagonal elements are shear stresses. The tensor is always symmetrical and positive definite for any physical material.

It turns out that stress tensors have the interesting property that it is always possible to rotate the coordinate system such that the tensor takes the simplified form:

$$\boldsymbol{\sigma} = \begin{bmatrix} \sigma_1 & 0 & 0 \\ 0 & \sigma_2 & 0 \\ 0 & 0 & \sigma_3 \end{bmatrix} \quad (5.3)$$

In this particular rotation of the coordinate system, the material experiences no shear stress. The normal stresses in this state,  $\sigma_1$ ,  $\sigma_2$  and  $\sigma_3$ , are called principal stresses, and  $\sigma_1 \geq \sigma_2 \geq \sigma_3$  by definition. Most people have an immediate physical comprehension of the difference between shear and normal deformation and are able to recognize these states when they see them applied to a soft material with sufficiently large deformations. It is mind-boggling that viewing the same material point in the same state of deformation from a different vantage point would reveal no shear. That is, however, a mathematical fact.

There exists another rotation of the coordinate system where the shear stresses are at their maximum, and it furthermore turns out that these shear stresses are equivalent to the so-called deviations of the principal stresses:  $\sigma_1 - \sigma_3$ ,  $\sigma_1 - \sigma_2$ , and  $\sigma_2 - \sigma_3$ . We can therefore conclude that there exists only a single state of stress that has no shear, namely the case where  $\sigma_1 = \sigma_2 = \sigma_3$ . This case of similar stress in all directions is called a hydrostatic

stress state because it is equivalent to the state of a material subjected to constant pressure from all sides as if it were submersed into water.

We can now look at the definition of von Mises stress in terms of principal stresses:

$$\sigma_{vM} = \sqrt{\frac{1}{2} \left( (\sigma_1 - \sigma_2)^2 + (\sigma_1 - \sigma_3)^2 + (\sigma_2 - \sigma_3)^2 \right)} \quad (5.4)$$

Notice that Equation (5.4) contains only the principal stress deviations. This means that

1. Hydrostatic pressure, according to this criterion, does not contribute in any way to the yield of a material.
2. Shear stress, measured in the direction where it is at its maximum, is actually the sole contributor to damage of a material.

### 5.1.2 Damage of Living Tissue

There is good experimental evidence [5] that deformation alone, as with engineering materials, can cause injury in the form of cell necrosis to living tissue, but a criterion for material damage similar to the von Mises yield criterion does not exist for living tissues.

It is also fair to state that living tissues from a material science point-of-view are very different from the underlying assumptions of ductility, homogeneity and isotropy necessary for the von Mises criterion. Furthermore, living tissues in a state of homeostasis have the ability to repair themselves, whereas a partial damage of an engineering material is permanent.

It would appear that there is no reason to presume that living tissue composed of cells and protein structures would be damaged by similar mechanisms as engineering materials. However, similarly to living tissue, engineering materials are only homogeneous on a macroscopic scale. Metals are composed of crystals, many alloys even of patterns of different crystals and, on a smaller scale, materials are made of discrete molecules and atoms. Any material is in fact a structure on a sufficiently small scale.

Living tissues also share a high resistance towards hydrostatic pressure with engineering materials. Marine animals can transition without tissue injury between the sea surface and great depth where the water pressure is significant.

On the other hand, it is also known from clinical practice that a deformation of sufficient size sustained for sufficient time will lead to tissue necrosis. This is the case for pressure ulcers, a serious tissue injury affecting individuals with reduced sensibility and/or inability to relieve pressure on the tissues,

such as paraplegics, diabetics and bedridden patients in general. The etiology of pressure ulcers is poorly understood [1], and this lack of understanding is an obstacle to prevention and treatment of pressure ulcers.

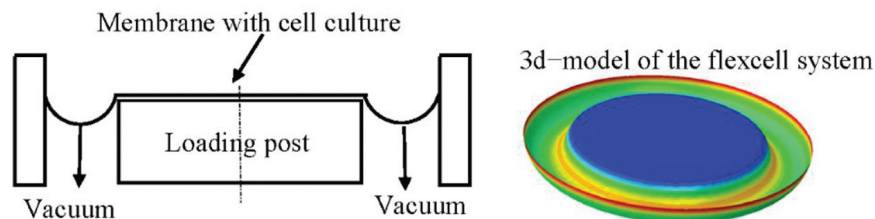
It is obvious that a closer investigation of the matter requires experiments in which living tissue can be subjected to controlled states of deformation. We shall base the following on strains rather than stress because strain is an experimentally measurable quantity and the strain tensor shares the aforementioned properties of the stress tensor, i.e. we can discuss normal strains, shear strains and principal strains.

## 5.2 Mechanical Loading in Two Dimensions

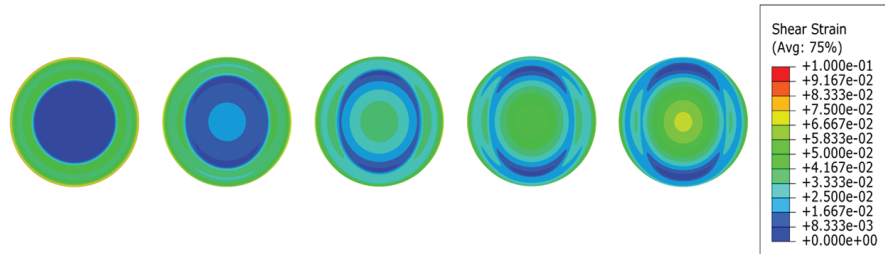
Mechanical stimulation of growing tissue in two dimensions can conveniently take place on elastic membranes, such as the Flexcell well system. This system comprises a circular silicone membrane on which tissue samples can be grown. The membrane can be subjected to deformation to impart strain on the attached tissue sample. The deformation is obtained by draping the membrane over a post by means of vacuum on one side of the membrane as illustrated in Figure 5.1.

Deformation experiments with the Flexcell system have traditionally taken place using circular posts. However, with non-circular posts, non-hydrostatic strain states can be obtained. It is possible by finite element analysis to obtain accurate predictions of the resulting strain states as illustrated in Figure 5.2.

Figure 5.2 shows the resulting maximum shear strains when draping a membrane over differently shaped posts ranging from circular (left) to elliptical with an axis ratio of 1.23 (right). Please notice that the circular post results in zero shear strain on the membrane over the post while non-circular posts impart shear strain to the membrane and thereby to the tissue sample.



**Figure 5.1** Draping the membrane over a post by means of vacuum. The colors on the right-hand picture are membrane shear strains simulated by a 3-D model. Notice that the strain field is uniform over the post.



**Figure 5.2** Finite element simulation of maximum shear strains on a membrane draped over posts with varying degrees of ovality.

While it is theoretically possible to control the amount of shear strain imposed on a tissue sample by means of non-circular posts, it unfortunately turns out that the adhesion between the silicon membrane and the tissue sample is insufficient to cause tissue injury. When imposing strain levels similar to those experienced by, for instance, muscle tissue in the buttocks in the seated posture, the cells come loose from the membrane and cease to follow the membrane's deformation. Thus, this method is not suitable for investigations of tissue injury, but it does allow for mechanical stimulation of tissue samples for other purposes [6].

### 5.2.1 Hertz-inspired Tissue Deformation

An alternative loading mechanism, inspired by Hertz contact mechanics [7], was invented to enable imposition of sufficient strain to cause necrosis. Mechanical problems of contact are in general highly nonlinear and very challenging, but a famous analytical solution attributed to Heinrich Hertz covers the special case of two linearly elastic spheres in contact. Given the material properties, the radii of the two spheres and the compression force, the analytical solution predicts the deformation state including strain and stress fields in the two parts. This is one of the truly classical problems of mechanics and it is an important part of the basis of the field of tribology and the ability to develop many important machine parts such as bearings and gears. An important special case of Hertz' solution is when one of the spheres has an infinite radius, i.e. is flat.

The case of a sphere pressed into a planar surface is axisymmetric and so is the resulting strain state. If we focus on the planar part, this means that the strain tensor in a given point depends only on the force (or relative displacement of the two parts), the point's depth under the planar surface

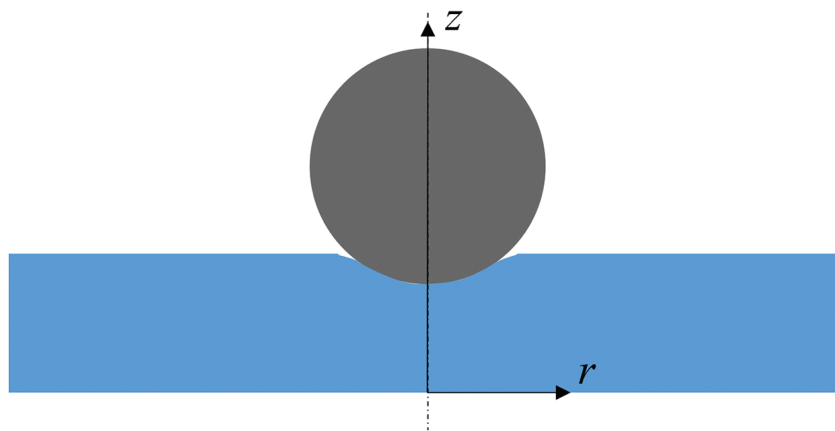
and the point's radial distance from the center of pressure as illustrated in Figure 5.3.

Thus, a cell embedded in the material under the flat surface will be subjected to a predictable strain state depending only on its location in the material. Furthermore, the strain state created by surface pressure is non-hydrostatic and three-dimensional, such that the strain ingredients that would be decisive to material yield or failure would be present in various amounts at different locations.

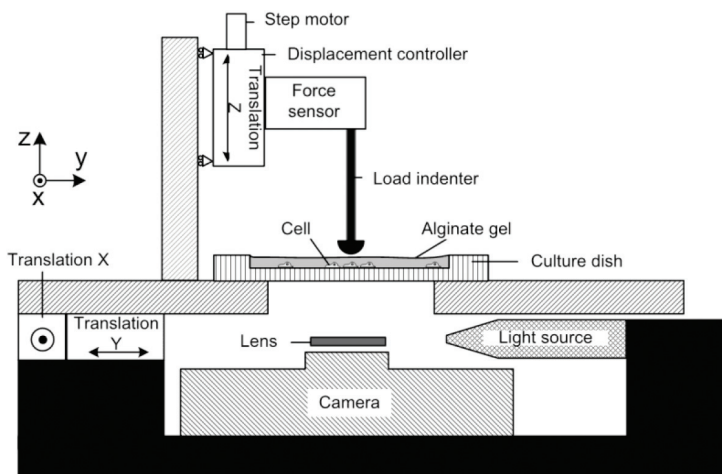
The feasibility of this idea depends on the material into which the cell is embedded being an order of magnitude stiffer than the cell, such that the deformation of the cell is commanded completely by the surrounding material. Furthermore, the material must sustain the life of the cell, i.e. be permeable to oxygen and provide nutrition. It was experimentally verified that specific compositions of alginate gels have these properties and can be exploited in the experimental setup illustrated in Figure 5.4.

In this setup, tissue in a culture dish is covered with a layer of gel and a spherical indenter controlled by means of precision motors can be pressed into the gel causing axisymmetric strain as described above.

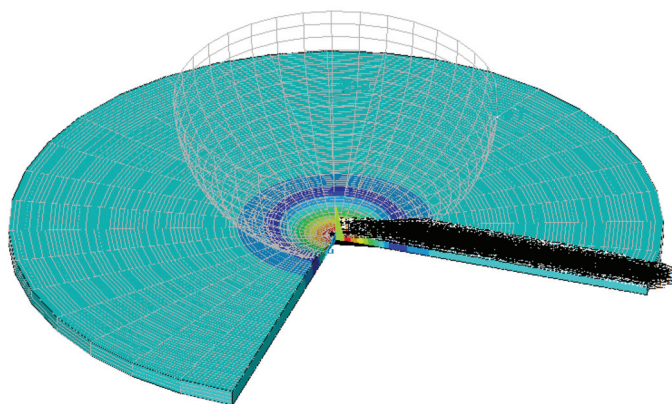
Hertz' surface pressure model requires linear elasticity of the involved materials, and this is not the case for a gel undergoing large deformations. The actual prediction of the strain field therefore cannot be based on Hertz' equations but must be computed numerically by a nonlinear finite element analysis as illustrated in Figure 5.5.



**Figure 5.3** Indentation of a sphere into a planar surface. The strain state will be symmetrical about the  $z$  axis, and the strain tensor at a given  $(r, z)$  position can be predicted.



**Figure 5.4** Experimental setup.

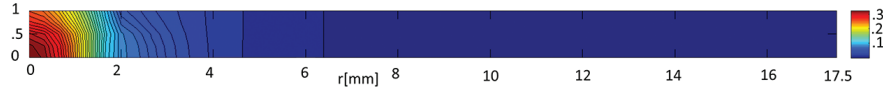


**Figure 5.5** Axisymmetric, nonlinear finite element model of sphere indentation into an alginate gel.

The finite element model predicts the strain field under the indenter and consequently also the strain state felt by a cell located at a given point in the gel. It is possible to visualize the strain field in terms of maximum shear strain to obtain the strain map of Figure 5.6.

The fact that the strain field decreases systematically (albeit nonlinearly) from the center of pressure to zero at about 8 mm distance means that the viability of cells under varying shear strain can be studied systematically using this setup.



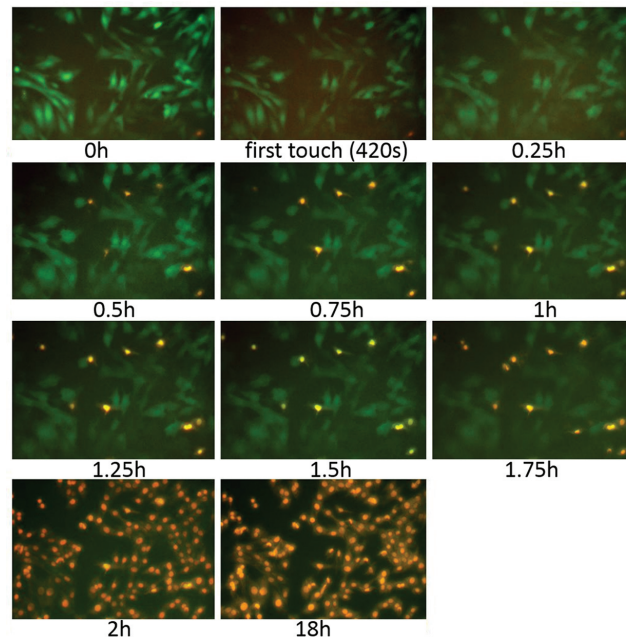


**Figure 5.6** Maximum shear strain as a function of radius ( $r$ ) and height ( $z$ ) in a gel under compression of a circular indenter. The strain is at its maximum at  $(r, z) = (0, 0)$  i.e. directly below the center of pressure and decreases radially.

The viability of the cells is observed under microscope by means of Lentiviral transfection of emerald green fluorescent protein (GFP). This staining technique allows the simultaneous study of cell morphology and viability by combination of the red and green channels of the microscope image. Necrotic cells appear as bright spots in the image.

### 5.2.2 Preliminary Results of Cell Straining

Figure 5.7 shows the viability of cells under the indenter, where the yellow dots designate dead cells. In this test, the indentation was increased over time to compensate for a significant stress relaxation of the gel.



**Figure 5.7** Necrosis of cells over time with different compression forces applied.

It is obvious that cell necrosis increases significantly over time until all cells are dead after about two hours. Please notice that it was verified prior to this experiment that no significant necrosis occurred under similar circumstances in the absence of the indenter load. Thus, the experiment corroborates the theory that imposed and sustained deformation causes cell necrosis.

This experiment simply observed cells directly under the indenter, while an attempt to determine the correlation between location, and thereby strain state, and the risk of necrosis is left to future studies.

### **5.3 Conclusions and Outlook**

A theoretical foundation addressing strain state and cell necrosis has been developed, and an experimental procedure capable of imposing controlled amounts of strain to cells imbedded into gel is established and tested. The tests show that the imposed strain has a clear influence on the necrosis of the cells, and it is possible that such experiments, when conducted systematically, can lead to development of an actual cell necrosis theory.

Such a theory, in turn, is of fundamental as well as practical interest. The clinical uses include prevention and treatment of pressure ulcers and tissue engineering in a broader sense.

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