DEPARTMENT OF BIOMEDICAL ENGINEERING

University of Strathclyde Engineering

# Biomechanical Biomarkers of Cancer

Carl Muscat

201450764

Supervised by Dr Phil Riches

Thesis submitted towards the partial fulfilment of

*MSc Biomedical Engineering*

Academic Year 2014/2015

# Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

The copyright of this thesis belongs to the author under the terms of the United Kingdom Copyright Acts as qualified by University of Strathclyde Regulation 3.50. Due acknowledgement must always be made of the use of any material contained in, or derived from, this thesis.

Signed: Date:

# Acknowledgements

It is only with the help and support from several people that the completion of this project was possible.

First and foremost I would like to take this opportunity to thank my supervisor Dr Phil Riches for his invaluable support, assistance and guidance throughout my project.

I would like to acknowledge Brian Cartlidge and thank him for the computational assistance in setting up the work station.

I would also like to recognise the work carried out by Busby and colleagues (2013), without which I would not have any reference to base my investigations on.

Additionally, I would also like to say a huge thank you to all my family. Not only for their continuing support and encouragement throughout my Undergraduate degree, but for being just as supportive this past year during my Masters Degree. A special mention must go to my mum, dad and brother without whose support I surely would not have been able to complete this year.

# Table of Contents

# List of Figures

# Abstract

Cancer has become one of the most common diseases, its treatment and diagnosis is of utmost importance. Because of this, it is important to find methods of diagnosing this disease at the earliest possible stage. This aim of this project is to assess the use of mechanical properties of cancerous cells for a new diagnosis method that would be faster, more reliable and would be able to identify the disease at a very early stage.

A model of cancer cells embedded in collagen hydrogel was created using finite element software and cancer cells were modelled using various different properties by varying parameters such as the size, depth, Young's Modulus, and hydraulic permeability. The effect of these parameters on the response was assessed by applying a ramp-hold loading and a sinusoidal load to the model, and then analysing the force and phase responses.

It was found that very small cancers cannot be diagnosed through this method. Diagnosis of more developed cancers having higher stiffness and/or lower permeability can be achieved by analysing the response of the system; however, it was noted that at high values of Young's Modulus, the hydraulic permeability did not affect the response by much, whilst at low values of Young's Modulus, the effects of hydraulic permeability were more visible in the response. It was also found out that the cancer cells should be placed as far as possible from the moving surface and that higher frequencies (when using a sinusoidal load) tend to give better results in terms of force response.

# Introduction

The human body is made up of various types of tissue, which consist of living cells that are embedded in an extracellular matrix (ECM). There are mainly four types of tissue in the body (Figure 1); nervous tissue, epithelial tissue, connective tissue, and muscle tissue. Each type of tissue has different mechanical properties due to different composition of substance in the ECM.

Nervous tissue encompasses all the structures that can transmit nervous impulses through the body. This includes the brain, spinal cord, and all nerves in the peripheral nervous system. Epithelial tissue normally refers to tissue that serves as a protective layer, such as the skin surface and the lining of the gastrointestinal tract. Connective tissue includes bones, tendons, fat, and any other type of structure that serves as a protective or padding structure such as cartilages. Muscle tissue can be subdivided into three further categories: cardiac muscle (the muscles of the heart), smooth muscle

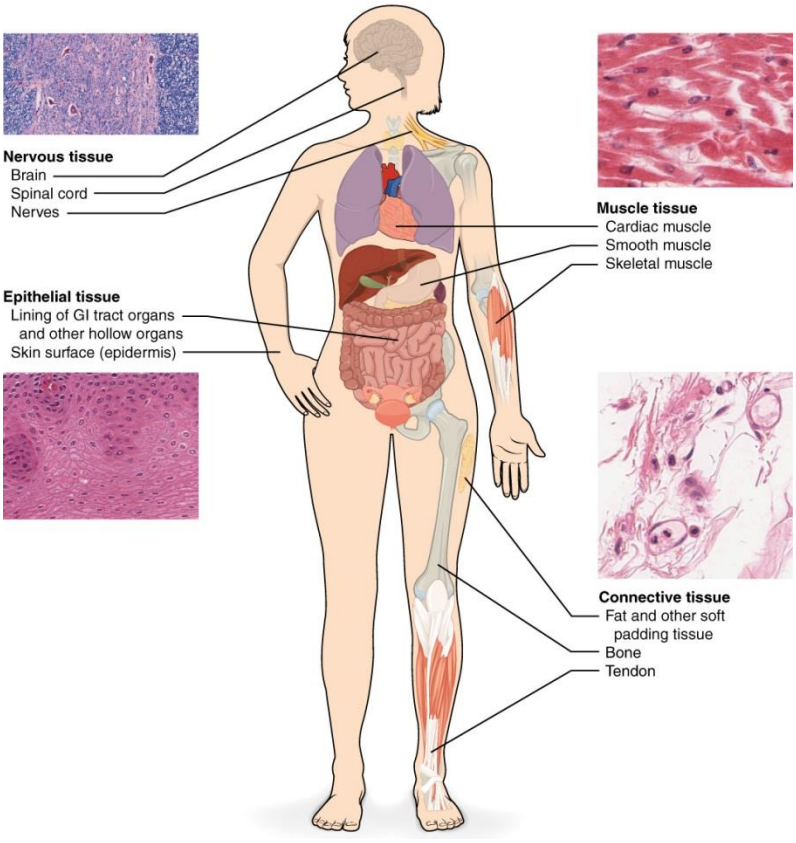


*Figure 1: The various types of tissue in the body*

(involuntary, non-striated muscles like the muscles in the gut), and skeletal muscles (the voluntary muscles that we consciously use to move around).

Cells in the tissues normally divide at a relatively slow rate, however, there can be a condition where cells become neoplastic such that protein synthesis of cells is altered. This causes them to start behaving and dividing abnormally. Such cells are called tumour cells. Tumour cells can be either benign or malignant. The term benign refers to tumour cells that remain localised and eventually stop dividing (this normally occurs due to a lack of space), and as such they are not dangerous to the body. Malignant tumours on the other hand are much more dangerous than benign tumours since they have the capability of invading new organs and due to this reason they can cause serious and extensive damage to the organs. The process of invading new organs is termed metastasis of the tumour and this causes what is known as cancer.

Cancer has become one of the most common and dangerous diseases nowadays in fact in 2011 in the UK there were 331,487 new cases of cancer diagnosed and 161,823 individuals lost their lives to this disease in 2012. Such a disease is very difficult to treat although there have been many improvements in treatment and in 2011, the survival rate was about 50%. The treatment of cancer is very much dependent upon its early diagnosis. The earlier the disease can be diagnosed, the easier it can be to treat the disease and the higher is the probability of survival after treatment. As such it is important to have good and accurate methods to diagnose the disease at its early stages in order to increase survival rate of the patients. (UK) (UK)

There are various methods in which cancers can be diagnosed. Usually the patient is first subjected to physical tests and the doctor checks the patient by manually feeling for lumps during a physical examination. Imaging tests such as magnetic resonance imaging (MRI) and computed tomography scanning (CT-scanning) amongst others may also be used in such a test in order to determine the presence of lumps in soft tissue. This however does not indicate whether the patient has cancer or not, since benign tumours are fairly common in the body and can also cause lumps. As such, after such testing, normally a biopsy is usually taken and a biopsy specimen is collected. This specimen can be further analysed using chemical tests and by observing under microscope so as to identify whether the patient has cancer or not. Such tests normally take a long time and this means that cancer cannot be diagnosed immediately on the spot. (Society)(Society)

There have been various studies in the past which indicate that cancerous tissues have different mechanical properties than normal tissue. This occurs mainly because such tissues have an ECM that has different properties than normal tissue. Cancerous cells are also characterised by higher plasticity and this also contribute to cancerous tissue having different properties. The aims of this project are:

- To use Finite Element Analysis (FEA) to create various models of cancerous tissue within a collagen hydrogel.
- To observe the effect that biopsy size has on the mechanical response of the model.
- To observe the effect that the stage and type of cancer have on the mechanical response of the model.
- To use the results from these Finite Element models to propose a new method for diagnosis which would ideally be more accurate and faster than the conventional methods.

# Literature review

## Cancer

Cancer is defined as a class of genetic diseases which result in the alteration of genetic pathways and their associated molecular pathways such that carcinogenesis occurs. These alterations lead to abnormal cell growth and have effects on cellular metabolism. Such cells have only two aims; to survive within the body, and to spread to other parts (metastasis). Cancer is caused by the formation of neoplastic cells (tumours) which then spread and expand to become malignant and form a cancer. The process of cancer formation is called carcinogenesis, and it can be split into two main phases: initiation and promotion.

Initiation refers to the initial formation of a neoplastic cell due to the effect of a carcinogen (a substance that causes formation). Carcinogens can in turn be of two main types: genetic and epigenetic carcinogens. Genetic carcinogens are capable of giving rise to electrophilic metabolites, which can in turn cause damage to macromolecules in the cell and affect its metabolism. Such carcinogens normally react with DNA to cause mutations and may include substances ranging from asbestos, to nuclear radiation. On the other hand, epigenetic carcinogens are not capable of directly causing mutations or affecting DNA in any way; however, they can cause hormonal imbalance, cytotoxicity and cell injury. These in turn affect cell behaviour and cause them to become neoplastic to form tumour cells and eventually become cancerous.

Promotion refers to the effect of a set of chemicals (promoters) that are not capable of causing neoplasticity and thus are not capable of causing cancer. However, these chemicals are capable of causing metastasis of tumour cells that can result in cancer. A dose of carcinogen that causes neoplasticity but no formation of cancer may be able to cause cancer in the presence of a promoter(Faguet, 2008; Ko, 2008) (Allan), 2007; Souhami and Tobias, 2007).

## Types of Cancer

There are various types of cancer; in fact, every cancer in any type of patient may be described as unique because the mutation that causes cancer is usually different in each case. Despite this, cancers may be classified using two main methods. They can be classified according to the organ in which they are start forming (an example of this is

the use of terms such as lung cancer, breast cancer, etc.). Cancers can also be classified according to the cell from which they originate as further described below (Barry, 2010; Ko, 2008) (Souhami and Tobias, 2007).

### Carcinoma

Carinoma is a type of cancer that starts with neoplasticity of the epithelial cells. These are cells that line the inner or outer surface of the body (e.g. skin, stomach walls, intestine walls, etc.). It is also the most common type of cancer and can be subdivided into other types according to the type of epithelial cells that it originates from (e.g. adenocarcinoma, basal cell carcinoma, etc.)(Ko, 2008) (Souhami and Tobias, 2007).

### Sarcoma

Sarcoma is a type of cancer that starts with neoplasticity of bone and soft tissues such as muscle, fat tissue, lymph and blood vessels. It can also originate from fibrous tissue such as cartilage and tendons. Similarly to carcinoma, sarcomas can be classified according to the specific cell type that they originate from resulting in terms like osteosarcoma (originates in bone), and liposarcoma (originates in fat) (Ko, 2008) (Souhami and Tobias, 2007).

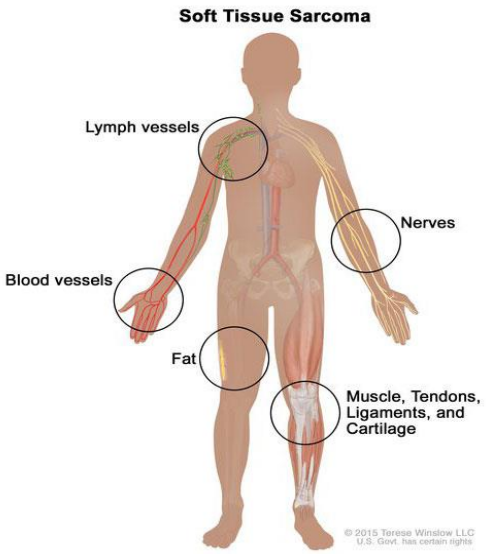


*Figure 2: Tissues from which sarcoma can originate*

### Leukemia

Leukemia is another type of cancer that starts with tissues in the bone marrow. They are different from other types of cancer in that they do not form solid tumours but cause the build-up of large numbers of neoplastic cells in the blood and bone marrow. This causes the absence of normal cells in these places, obstructing the uptake of oxygen and the fighting of infections and/or diseases (Ko, 2008).

### Lymphoma

Cancers that arise from lymphocytes are called lymphomas. In this type of cancer, abnormal lymphocytes build up in lymph nodes and lymph vessels and it can cause deficiencies in the disease fighting mechanisms of the body. There are two types of

lymphoma; Hodgkin lymphoma and non-Hodgkin lymphoma (Ko, 2008) (Souhami and Tobias, 2007).

### Multiple Myeloma

Multiple myeloma is a type of cancer that originates from another type of immune cells called plasma cells. These abnormal cells (called myeloma cells) build up in the bone marrow and can form tumours in bones all around the body(Faguet, 2008; Ko, 2008) (Souhami and Tobias, 2007).

### Melanoma

Melanoma develops from ells called melanocytes that are responsible for making pigments in the body. The most common type of melanoma is found on the skin; however, other types can form on other parts of the body that contain pigments like the eyes (Faguet, 2008; Ko, 2008) (Souhami and Tobias, 2007).

### Brain/ Spinal Cord Tumours

Brain and spinal cord tumours are tumours that originate in cells within the central nervous system (CNS) and they can be further categorised according to the type of cell from which they originate. Like other tumours, they can be benign or malignant, where the malignant type can be very dangerous and complex to cure (Faguet, 2008; Ko, 2008) (Souhami and Tobias, 2007).

### Other tumours

Other types of tumours may include germ cell tumors, which are tumors that start in the cells that produce gametes through meiosis. Neuroendocrine tumors form from cells that release hormones. This may cause alterations in hormonal level within the body, thus altering body function. Carcinoid tumors are yet another type of tumor. They are slow-growing tumors that are usually found in the gastrointestinal system. Like all other tumors, all of the tumors mentioned above can be benign or malignant (Faguet, 2008; Ko, 2008; Souhami and Tobias, 2007).

## Grading of Tumours

Cancerous cells are different from normal cells. The degree of variation between cancerous cells and normal cells is termed as the grade of a tumour. If cells in the tumour are very similar to normal cells, they tend to be well differentiated and will

have a tendency to divide at a slower rate than tumours that are highly dissimilar to normal cells (which also tend to be undifferentiated to a high degree). Because of this, cancerous cells that are very similar to normal cells are referred to as low grade cancers, whilst cancerous cells that are different from normal cells to a high degree are referred to as high grade cancers.

The grade of a cancer is normally identified by taking a biopsy and examining the tumour cells under microscope. There are various grading systems that can be used and these depend on the type of tumour and location. Tumours that do not have a specific grading system are graded by using numbers from 1 to 4, where a Grade 1 tumour refers to a tumour that is highly differentiated and very similar to normal cells, whilst a Grade 4 tumour refers to a tumour that is very different from normal cells and tends to divide at a very high rate. This means that Grade 4 tumours are much more dangerous than Grade 1 tumours and have a larger tendency to form cancers (Faguet, 2008; Ko, 2008) (Souhami and Tobias, 2007).

## Staging of Cancer

Staging is a method used to describe the severity of a cancer. It is based on the size of the original tumour and whether or not it has spread throughout the body. In order to stage a cancer, various elements are considered. These include:

- Size and the cell type from which the initial tumour developed.
- Tumour size and extent.
- The degree of spread of cancerous cells to nearby lymph nodes in the body.
- Number of primary and metastatic tumours together with the degree of metastasis.
- Grade of the tumour.

Staging can be done using various methods; however, it is usually divided into five categories as shown in the table below. The TNM system is also another commonly used method of staging and most of the designations by the TNM system can also be allocated to one of the five stages (Ko, 2008; Souhami and Tobias, 2007).

| **Stage** | **Definition** |
|---|---|
| Stage 0 | Localised cancer of a small size with no metastasis. |

| | |
|---|---|
| Stage 1, Stage 2 and Stage 3 | Cancer that is of a larger size with a certain degree of metastasis. Larger stage numbers indicate a larger size of the initial tumour or a higher degree of metastasis and/or spread within the body. |
| Stage 4 | Cancer that has spread to areas of the body that are very distant from the original tumour. |

## Diagnosis of Cancer using a Biopsy

Diagnosis of cancer is normally carried out in two main phases. In the first part, a doctor carries out a physical exam on the patients where lumps in the body can be identified. These may potentially be cancers. Sometimes, imaging techniques are also used in order to find lumps that are located deeper in tissue and that cannot be physically located. The presence of such lumps does not always indicate the presence of cancer; in fact, many of these lumps can be the result of inflammation or benign tumours. This leads to the need of taking a biopsy in the second part of diagnosis. This helps the doctor identify whether the identified lump is a cancer or not by removing the lump completely or by taking a sample of cells from the lump. The taken biopsy is then further tested in order to identify whether cancer is present or not. There are various methods of taking a biopsy specimen from a patient. The method used is dependent on the location and the suspected type of the cancer. Some of the methods of taking biopsies are described below.

### Fine Needle Aspiration (FNA)

FNA is a technique that enables the extraction of fluids and small amounts of tissue through a very thin, hollow needle attached to a syringe as shown in Figure 3. While taking the biopsy, the doctor can aim the needle while feeling the tumour (lump) if it is located close to the skin surface. If the tumour is not near the skin surface, imaging techniques such as MRI and/or ultrasound can be used to guide the needle precisely to the location of the tumour. The main advantages of FNA are that it is not highly

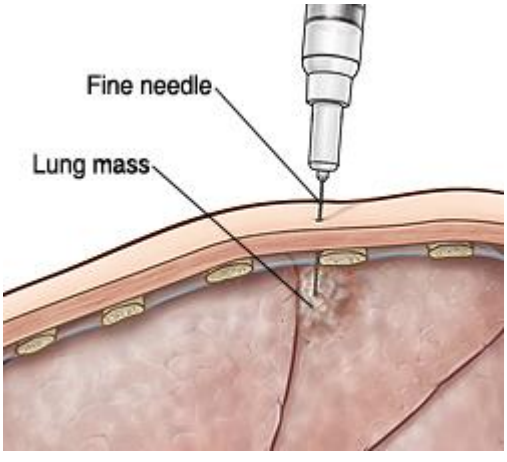

*Figure 3: Schematic diagram of FNA*

intrusive since skin does not need to be opened and that diagnosis can often be made the same day that the biopsy is taken. On the other hand, in some cases, the amount of tissue extracted may not be large enough to diagnose the cancer. It is important to note that FNA can also be used for cytology tests since it is capable of obtaining fluids from the body. (Orell, 2012)

### Core Biopsy

Core Biopsy is a technique that is very similar to FNA in that it also involves the use of a needle to obtain the biopsy sample; however, in this case, the size of the needle is larger than that used in FNA and it requires local anaesthesia. Like FNA, the needle can be guided and aimed while feeling the tumour manually or by using imaging techniques as required. Sometimes special suction devices (using vacuum) can also be used in order to obtain biopsies of larger size. It is also important to note that the processing time for core biopsies is usually longer than FNA and as such, more time is required between the time of biopsy and diagnosis results .

### Excisional/Incisional Biopsy

An excisional/incisional biopsy can be carried out by cutting the skin in order to remove the whole tumour (excisional biopsy) or part of it (incisional biopsy). Normally, local anaesthetics are used in these cases; however, if the tumour is located in the chest or abdomen, a general anaesthetic is used. Using these excisional/incisional biopsies, the obtained biopsy sample is of much larger size.

### Endoscopic biopsy

An endoscopic biopsy is a biopsy that is obtained through an endoscope. The endoscope is normally used to enable doctors to look inside different parts of the body; however, it also has the capability of taking out small tissue samples that can then be further analysed and tested.

### Other types of biopsies

There are various other types of biopsies that can be taken like laparoscopic biopsies, thoracoscopic and mediastinoscopic biopsies. These are all similar to endoscopic biopsies in that a similar device to an endoscope is used; however, an incision needs to be made in order to insert the device and observe the organ of concern and take

biopsies. Laparoscopic biopsies are taken from the abdomen, whilst thoracoscopic and mediastinoscopic are taken from the chest area (Ko, 2008).

## Processing and testing of biopsies

After obtaining the biopsy specimen, the sample is usually stored in a container having a mixture of water and formaldehyde (or any other substance used for preserving). At this point it is labelled with the place it was taken from and patient details. Subsequently, the sample is visually observed (gross examination) in order to notice features that might diagnose cancer by using the naked eye. In some cases photos of the specimen are also taken so that further examination can be done.

In the case of smaller specimens, such as those obtained from FNA and core biopsies, gross examination is not carried out since these are too small to observe visually with the naked eye. In this case, the specimen is observed under light microscope. This is done by putting the sample into small containers called cassettes. These hold the sample securely whilst it is being processed so that it can be observed. This processing usually takes several hours and is generally carried out overnight. After processing, the samples are put in a mold containing hot paraffin wax, which solidifies around the specimen and encases it. The specimen is then cut using microtome blades and is stained using several dyes so that certain structures are enhanced and it can be viewed under light microscope. This technique is also used for larger biopsies when gross examination is not sufficient for accurate diagnosis. When observing the specimen under light microscope, the doctor looks for specific signs of cancerous tissue such as the size and shape of the cells and their nucleus, the arrangement of cells and the grade of the cancer. These factors help to diagnose whether a tumour is benign or malignant.

## Tests used to diagnose cancer

### *Histochemical staining*

Histochemical staining involves the use of chemical dyes to identify certain substances that are found in cancerous cells. The presence of such a substance can be identified through the use of this dye and consequently the type of cancer can be diagnosed. An example of this is the use of the dye mucicarmine on a biopsy specimen taken from the lung. This dye is attracted to mucus and stains pink upon contact with it. Thus, the use of such a stain can identify the presence of cells that can produce mucus. This

means that if pink stains are observed under light microscope, the type of cancer is an adenocarcinoma, since this type of cancer cells can produce mucus.

### *Immunohistochemical staining*

Immunohistochemical staining involves the use of antibodies in order to identify whether cells are cancerous or not. In normal circumstance, the body produces antibodies in order to fight bacterial infections. However, in this case, special antibodies are created in the laboratory and these are used to identify cancerous cells. Antibodies are proteins that are attracted to a specific substance (antigen) within a cell and eventually bind to it to disable the cells. The antibodies created in the laboratory bind to specific substances that are found in cancerous cells only and they stain these cells to a particular colour. Cancerous cells can subsequently be identified according to the antibody that was used and by examination under light microscope, where the cancerous cells will be stained to a different colour.

### *Electron microscopy*

Normally, biopsies are observed under light microscope; however, sometimes this is not enough to identify the type of cancer. Histochemcial and immunohistochemical tests are also inconclusive sometimes. In such cases, an electron microscope is used to identify the type of cancer that is present in a biopsy. This microscope has a much larger magnification capability than a light microscope and can be used to identify structures that are related to a particular type of cancer. An example of this is in the case of melanomas; the electron microscope is used to identify structures called melanosomes within the cells. These structures can only be found in melanomas and thus, the presence of this type of cancer can be confirmed by identifying such structures.

### *Flow cytometry*

Flow cytometry is a type of test that uses the same principle as immunohistochemistry. Antibodies are used to attach to cells; however, these antibodies do not stain the cells under normal light. The biopsy specimen is then suspended in fluid and passed under a special laser that makes the cells with antibodies attached emit light. The emitted light is then analysed using a computer and conclusions about the cancer can be made. In most cases, flow cytometry is used to diagnose lympohomas and leukemias. If the

same antigens are detected on a variety of cells, there is a higher likelihood of leukemia or lymphoma. If there is a variety of antigens that are detected on the cells, it is less likely that the cancer is leukemia or lymphoma.

Flow cytometry can also be used to analyse the amount of DNA in cells within the biopsy specimen. This is done by using special dyes that react with DNA instead of antigens. A cell that has a normal amount of DNA is called a diploid cell, whilst a cell that has a larger amount of DNA is called an aneuploid cell. Aneuploid cells tend to have a higher growth and spread rate than diploid cells. Thus, aneuploid cancers tend to be more aggressive and spread faster. Another use of flow cytometry is to determine the amount of cells that are in the S-phase. The S-phase is a phase of the cell cycle where cells divide. Thus, the amount of cells in the S-phase is directly related to the growth rate and spread of the cancer.

### *Image cytometry*

Like flow cytometry, image cytometry is used to determine the amount of DNA in cells. DNA stains are also used; however, instead of suspending the biopsy in fluid and using a laser beam, a digital camera in conjunction with a computer is used to analyse the results.

### *Cytogenetic tests*

In cytogenetic tests the cancer cells are grown in culture for about two to three weeks. After this, they are observed under light microscope to identify abnormalities in their chromosomes. Cytogenetic tests can also help in predicting the cure that should be used for a particular cancer and they are particularly useful in the diagnosis of leukemias, lymphomas and sarcomas.

### *Fluorescent in situ hybridisation (FISH)*

FISH is used to identify abnormalities in the chromosomes of cells. In this case, dyes that attach to certain genes (parts of the chromosome) are used. Thus, abnormalities like translocations and duplications can be identified and the type of cancer can be specified. FISH is also used to predict whether certain drugs could be useful in the treatment of certain cancers. For example in breast cancer, FISH can be used to detect if there is an abnormal amount of HER2 genes and this can help the doctors in selecting an appropriate treatment.

### *Polymerase chain reaction (PCR)*

PCR, also known as reverse transcriptase PCR (RT-PCR) is a very sensitive molecular test that is capable of identifying specific strands of RNA that is used to synthesise particular proteins. A different RNA molecule is present in our body for every protein that needs to be synthesised. Since cancerous cells are capable of creating abnormal amounts of proteins, this means that abnormal amounts of RNA strands are present in the cells. These abnormal RNA strands can be identified using PCR. The most advantageous point of PCR is that it can be used to detect very small amounts of cancerous cells that would otherwise be missed using other tests. On the other hand, this may serve as a deterrent since the presence of a small amount of cancer cells in the bloodstream and lymph nodes is not always indicative that a cancer will metastase to a degree that it will affect survival. PCR can also be used to predict whether a cancer is very aggressive or not according to the levels of important RNA strands that have been detected.

### *Gene expression microarrays*

This type of test involves the use of a very small device that can compare the relative levels of hundreds or thousands of RNA strands. The levels of RNA strands in a cell can indicate whether a cancer is present or not together with the type of cancer and its classification. Such tests can also help the doctor in the prescription of appropriate drugs for cure.

Gene expression microarrays are very useful when a cancer has spread to multiple locations and the primary source of the cancer is unknown. The patterns of known types of cancer can be compared with the test results in order to determine the primary source of the cancer.

### *DNA sequencing*

DNA sequencing is a test that is used to identify mutations in DNA that might lead to the development of cancerous tissue. After mutations have been identified, drugs can be prescribed by a doctor in order to reduce the expression of the mutated genes or to help with the condition that the cancerous tissue is creating(Ko, 2008; Society).

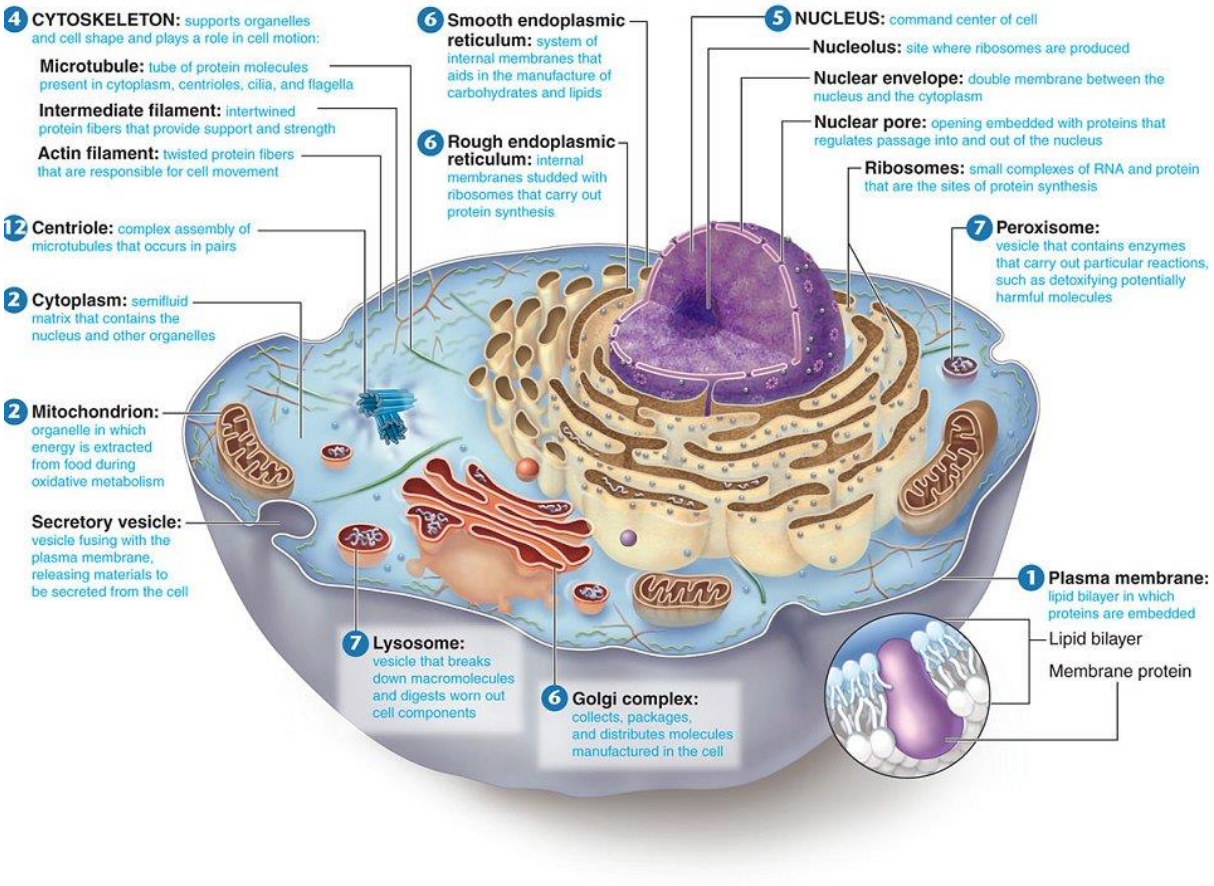


*Figure 4: The structure of a normal cell together with its organelles and their function.*

## Molecular biology of cancer and its relation to the mechanical properties

As mentioned previously, cancer is a disease that is characterised by the abnormal behaviour of cells. Like other normal tissue, cancerous tissue is made up of cells embedded in an ECM. Cells bind to the ECM by using the integrin receptor in their cell membrane. As shown in Figure 4, a normal cell is made up of organelles in a cytoskeleton.

### The cytoskeleton

The cytoskeleton is the main structure that contributes towards the mechanical properties of the cell. It is made up of three distinct types of polymer biomolecules; actin filaments, intermediate filaments and microtubules, all of which contribute towards the structural properties of the cytoskeleton.

#### *Actin filaments*

Actin microfilaments are reversible assemblies of actin monomers (F-actin and G-actin intertwined as shown in Figure 5) and they are attached to the phospholipid

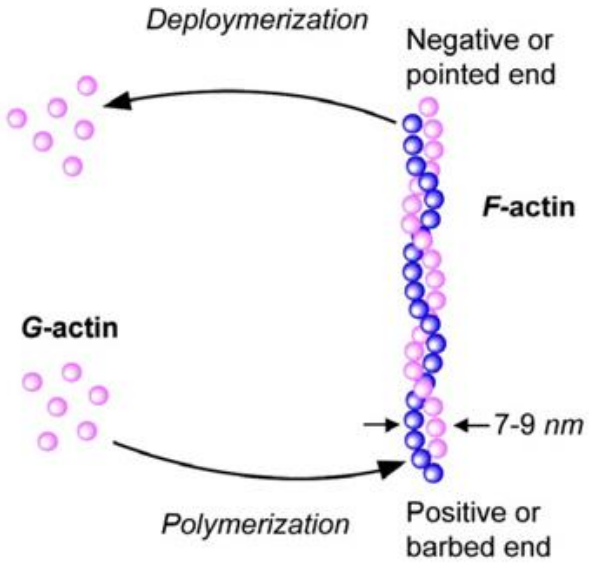


*Figure 5: The structure of Actin filaments*

membrane (plasma membrane). These filaments tend to form when the cell requires additional strength and they have a Young's modulus of 1.3-2.5 GPa. They also exhibit viscoelastic properties which vary according to the concentration of their biopolymers (Suresh, 2007; Wenwei Xu, 2012).

### *Intermediate filaments*

Intermediate filaments can be made up of various proteins (like keratin and vimentin). The protein is coiled with other strands (of the same protein) to form intermediate filaments as shown in Figure 6. Intermediate filaments are compliant enough to allow

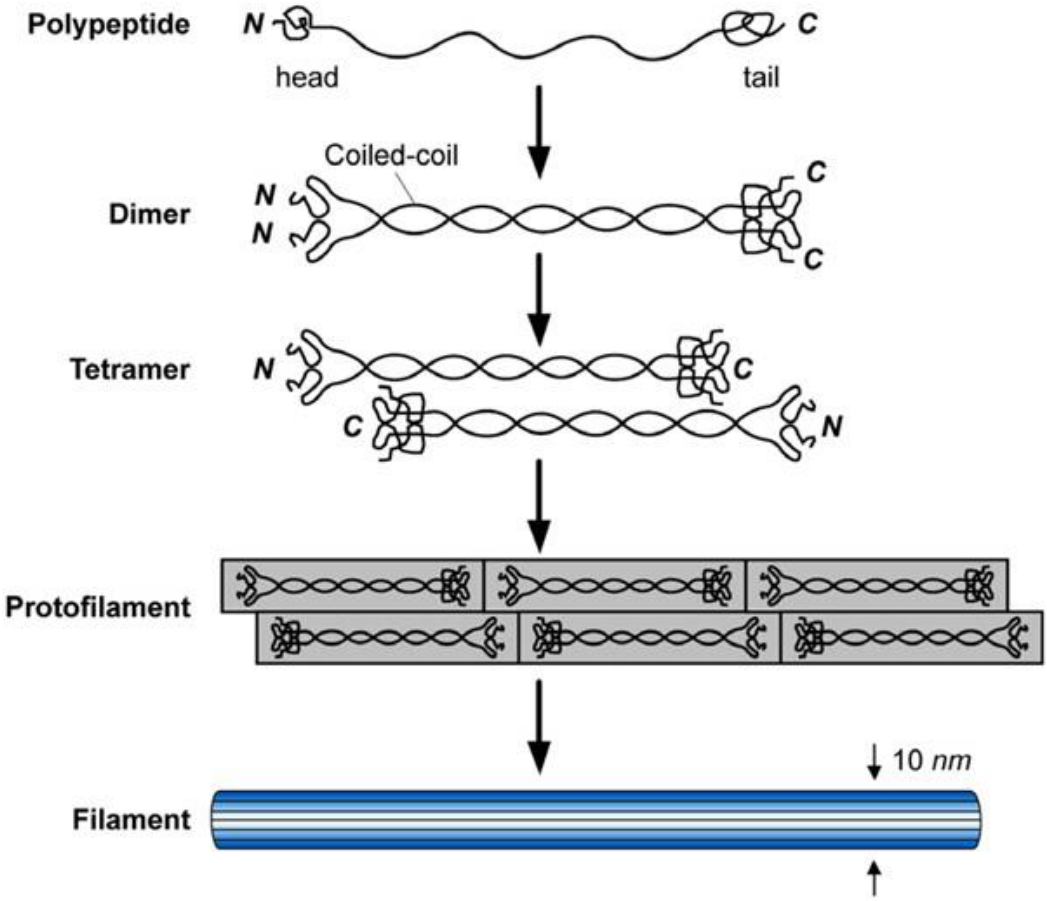


*Figure 6: The structure of intermediate filaments*

moderate deformation of the cell without damaging the organelles. They also provide enough rigidity for structural integrity of the cell and to resist shear deformation. Intermediate filaments also have the capability of strain hardening in order to bear stresses at strains where actin filaments lose their structural integrity (Suresh, 2007; Wenwei Xu, 2012).

### *Microtubules*

Microtubules are made of tubulin forming a hollow rod of outer diameter 25nm and inner diameter 14nm. The tubulin dimers polymerise to form tubules as shown in Figure 7. The filaments are characterised by an end that grows rapidly and another end that grows slowly. Microtubules do not contribute greatly towards the mechanical properties of the cell; however, they help in determining the shape of the cell.

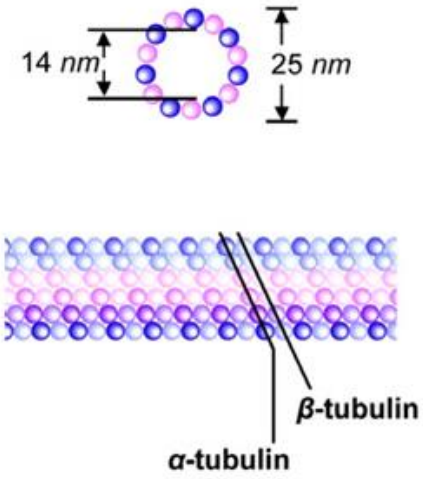


*Figure 7: The structure of microtubules*

In cancerous cells, there are defects in the structure of the above mentioned structures in the cytoskeleton. These cause the cytoskeleton to have different mechanical properties resulting in a higher deformability of cells and a lower overall stiffness. These properties in turn affect the chemical reactions inside the cell. (Suresh, 2007; Wenwei Xu, 2012)

### The Extracellular Matrix

The ECM is the non-cellular component of tissue. It provides a means of scaffolding for cells and enables contact between individual cells and with the external environment (through integrin receptors). The properties of the ECM are important because they affect cell differentiation and morphology.

The ECM is mainly made up of proteoglycans and fibrous proteins. Proteoglycans fill in the interstitial spaces of the ECM and have the function of hydrating the structure, provide binding and also provide some of the force-resisting properties of the ECM. There are three main fibrous proteins found in the ECM; collagens, elastins, and fibronectins. The most abundant protein of these is collagen (type 1). Collagens are the main component which provide tensile strength to the ECM and also regulate cell adhesion. It is found as fibres within the ECM (Christian Frantz, 2010).

In cancerous tissue, the altered cytoskeleton of the cells causes the collagen fibres found in the ECM to form cross-linkages. This in turn gives rise to a higher overall stiffness of the ECM in cancerous cells. Such findings have also been proven by other studies. (Cox and Erler, 2011; Fenner et al., 2014; Kandice R. Levental, 2009; Lu et al., 2012)

## Modelling of soft tissue

It has been demonstrated by Busby et al. (Busby Graham A., 2013) that collagen hydrogel having 0.2-0.4% collagen can be appropriately modelled using the poro-elastic theory. Such gels are much more compliant and permeable than soft tissue, however, they are thought to be a good scaffold for tissue engineering to eventually become soft tissue, and so, they can be used in the culture of cancer cells. Since the ECM of cancerous tissue is stiffer than that of normal tissue, cancerous tissue is said to be of higher stiffness than normal tissue, despite the fact that cancerous cells exhibit lower stiffness and higher deformability(Baker et al., 2010; Butcher et al., 2009; Fenner et al., 2014; Lu et al., 2012). This means that cancerous tissue could be modelled as a collagen hydrogel; however, this model should have a higher stiffness of collagen (type 1) fibres (due to the cross-linking of fibres).

Since cancerous tissue can be modelled as a collagen hydrogel, it is of great importance to know some of the mechanical properties of collagen. It is also important to note that the elastic properties of cancerous cells are time-dependent. This means that such cells should not be modelled as an elastic medium, but a material model that incorporates time-dependence should be used. Two commonly used models are the visco-elastic and the poro-elastic models(Casares et al., 2015; Forgacs et al., 1998; Moeendarbary et al., 2013; Morgan et al., 2006; Ragsdale et al., 1997).

### Collagen hydrogels

Busby et al. (Busby Graham A., 2013) demonstrated that soft tissue can be modelled under confined compression as a collagen hydrogel having 0.3% collagen with the collagen fibres having an aggregate modulus $H_A = 0.9642MPa$ and a hydraulic permeability $k = 100\, mm^4/Ns$. It was also demonstrated that a poro-elastic model (biphasic) describes the collagen hydrogel with sufficient accuracy. It is also important to note that in confined compression, as in this case, the aggregate modulus ($H_A$) is used; however, this can be related to the to the Young's modulus ($E$) through the Poisson's ration ($v$) which was found to have a value of 0.125. (Farrell, 2012)

#### *Biphasic Theory and poro-elastic theory*

The poro-elastic theory was initially developed to describe soil mechanics (Mow, 1980). This theory was then used to develop an alternative formulation based on the

theory of mixtures (Prendergast P. J., 1996) so that it could be applied to articular cartilage and soft tissues. This was called the biphasic theory.

In the biphasic theory, the material is assumed to exist in two phases at the same time; a solid phase and a fluid phase. Each point in the tissue is taken to have both solid and fluid elements and transient stress and strain gradients can be predicted within the material. A biphasic material is said to be a deformable, permeable, porous solid matrix with an interstitial fluid.

It has been demonstrated that collagen hydrogels may be considered as biphasic(Busby Graham A., 2013). These may be considered to have a network of collagen fibrils representing the solid phase, which is filled with a large excess of interstitial fluid demonstrating the fluid phase. The fluid phase of collagen hydrogels is typically more than 99.5%. (Busby Graham A., 2013)

In the linear biphasic theory described by Mow et al. (Mow, 1980), the derivation describes the flow of fluid through the solid phase; however, one may note that there is no description of the pores or their size. Fluid flow through a porous solid is described using Darcy's Law with the equation:

$$w = -k\frac{\partial P}{\partial x}$$

Where $w$ is the discharge per unit area, also known as the flux or the flow rate in $m/s$; $k$ is the hydraulic permeability in $m^4/Ns$; and $\frac{\partial P}{\partial x}$ is the pressure difference across a distance $x$ given in $Pa/m$. It is also known as the pressure gradient. Darcy's Law illustrates that fluid flows down a pressure gradient, from a high pressure to a low pressure area. The flow of the fluid is governed by the hydraulic permeability, which describes the ability of the fluid to flow through the porous medium.

For the linear biphasic model, in confined compression (i.e. prevention of lateral expansion/contraction) the stress can be described as a linear function of strain using the equation:

$$\frac{\partial u}{\partial t} = kH_A\frac{\partial^2 u}{\partial x^2}$$

Where $H_A$ is the aggregate modulus in $Pa$, and it is defined as the stiffness modulus in confined compression; and $u$ is the displacement of the solid phase in the direction $x$ at time $t$.

The aggregate modulus can be related to the Young's modulus through the equation:

$$H_A = \frac{E(1-v)}{(1+v)(1-2v)}$$

Thus, only the Young's modulus is needed to create a biphasic model of soft tissue in this case, since the aggregate modulus can be subsequently calculated if the Poisson's ratio ($v$) is assumed to have a value of 0.125 (Busby Graham A., 2013; Karol Miller, 1998).

### Finite element analysis (FEA)

The finite element method is a numerical technique used to approximate a solution for an entire model by splitting the defined structure into a finite number elements. Elements are connected to each other at specific points called nodes. The response of each element is expressed at a set of nodal points by creating a system of differential equations for the structure. Each elemental solution is obtained with respect to adjacent elements until ultimately an approximate solution for the whole model can be obtained. The FE method is particularly useful when dealing with complex geometries like those found in biomechanical systems. This is because by using standard mechanics of materials theorems, such problems cannot be solved. Such a model provides the means of solving such problems by providing an approximate response of a complex system from the individual contributions of each element. (Maas S.A., 2012 ; Simon, 1991; Tavel, 2011 )

Finite element models typically comprise a large amount of nodes and thus the amount of differential equations and their complexity is very high. For this reason, special software is normally used in order to solve these equations (e.g. ANSYS, ABAQUS, FEBio, etc.). Such software normally has pre-defined equations incorporated into it that describe the material properties and normally there are also special algorithms for defining the positions of nodes and element shapes.

## Biomarkers

A biomarker is defined as a biological measure of biological state. It is a characteristic that can be used to identify the state of a particular biological function ranging from normal functions to pathogenic functions and can also include therapeutic and pharmacological functions. There is a wide variety of biomarkers that can range from genetic biomarkers and chemical biomarkers to physical biomarkers.

This means that the term “biomechanical biomarkers of cancer” refers to a biomarker that identifies the biological state of cancerous cells by using mechanical cues that indicate the development, stage, metastasis and location of the cancer amongst other properties (Mayeux, 2004; Tavel, 2011 ).

# Objectives

The objective of this project is to simulate a situation where cancerous biopsies are cultured in a collagen hydrogel so that the effect that stage of development of cancer can be modelled by varying the material properties of the geometry representing cancer.

Such a simulation will be done by modelling the geometry representing cancer with different material properties than those representing the collagen hydrogel. The parameters of interest in this case are:

- The size of the cancer – varied by increasing and decreasing the volume of material with different structure.
- The stage and grade of the cancer – varied by changing the stiffness and hydraulic permeability of the material with a different structure.
- The height of the culture within the structure will also be analysed in order to obtain the ideal position where the culture should be placed.

Different loading profiles will also be used in order to be able to fully characterise the properties of the cancer. The two profiles that will be used are a ramp-hold loading profile and a sinusoidal profile. The hypotheses in this case are:

- The properties of cancer can be assessed by analysing the peak force and equilibrium force response during a ramp-hold loading profile.
- The properties of cancer can be assessed by analysing the peak force and phase lag whilst using a sinusoidal loading profile.

# Methodology

## Experimental overview

As described in the literature review, cancerous tissue is characterised by an overall higher stiffness, which results in a larger Young's Modulus ($E$) and a lower permeability ($k$). Because of this reason, it was decided that the cancerous tissue in a soft collagen hydrogel would be simulated as a sphere in a cube (both consisting of

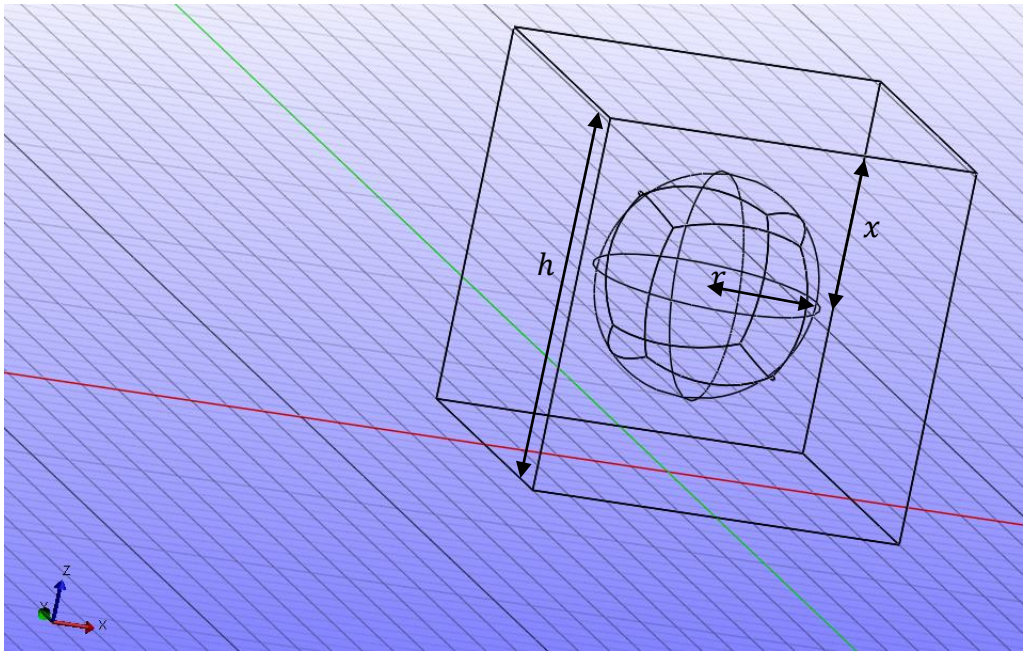


*Figure 8: Modelling of the actual situation*

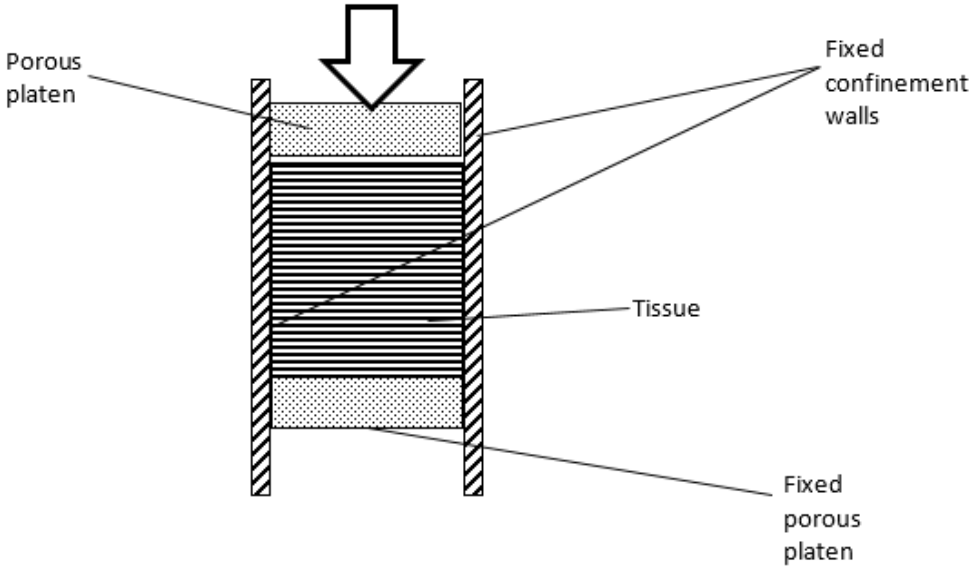


*Figure 9: Schematic diagram of confined compression*

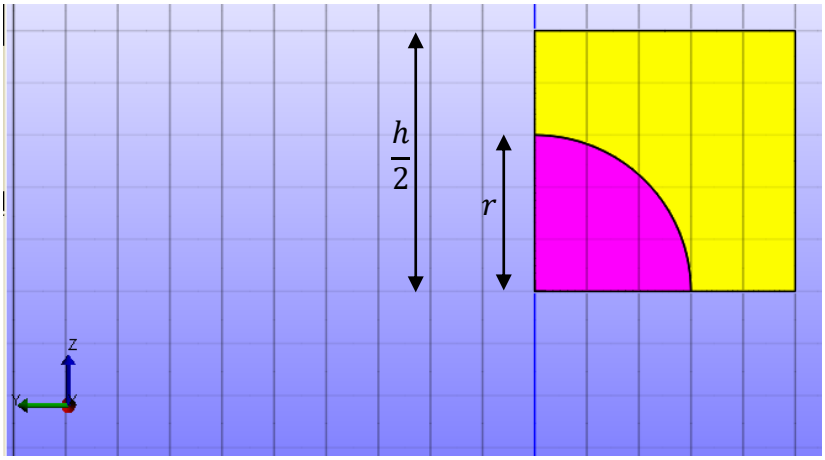


*Figure 10: Modelling the problem using the symmetric boundary conditions when the sphere is at the middle of the cube (x/h=0.5)*

biphasic material) as shown in Figure 8 and it was loaded in confined compression as described by Figure 9. The sphere represents the cancerous tissue and has different properties than the collagen hydrogel, which has $E = 0.9642MPa$ and $k = 100mm^4N^{-1}s^{-1}$ (Busby Graham A., 2013)

Various studies were carried out in order to examine the effect that a change in parameters has on the response of the described system:

In the first study, the material properties of the sphere representing the cancerous tissue were varied by increasing the Young's Modulus and also by decreasing the permeability in orders of 10. Other cases were also added when varying the Young's Modulus because after exceeding a value of 9.642MPa, the sphere started exhibiting a behaviour that was similar to a rigid sphere rather than cancerous tissue. This meant that the response for Young's Modulus between 0.9642MPa and 9.642MPa should be analysed in further depth, so, within this range, the Young's Modulus was also varied from value of 1MPa to 9MPa in steps of 1MPa. Such situations represent the different grades of cancer, where higher grades of cancer are simulated with a biphasic material that has a higher Young's modulus and a lower hydraulic permeability. This was done by varying one parameter at a time and keeping the parameters $r/h$ and $x/h$ constant at values of 0.3 and 0.5 respectively. This situation is symmetrical, thus, it can be modelled using finite element analysis by only modelling $1/8^{th}$ of the actual problem (as shown in Figure 10).

In the second study, the radius of the sphere was varied in order to observe the effect that the size of the tumour has on the response of the whole system. This represents a situation where the stage of cancer might be variable such that higher values of $r/h$ represent a cancer of higher stage. The study in this case was done by keeping the parameter $x/h = 0.5$ and also keeping the material properties of the sphere constant with Young's Modulus of $96.42MPa$ and Hydraulic Permeability of $10mm^4N^{-1}s^{-1}$ . This situation is symmetrical, thus it can be modelled using finite element analysis by only modelling 1/8$^{th}$ of the actual problem (as shown in Figure 10).

In the third study, the depth of the cancerous tissue was varied. This was done by changing the parameter $x/h$ whilst keeping the other parameters fixed such that $r/h = 0.3$, Young's Modulus of the sphere at a value of $96.42MPa$, Hydraulic Permeability of the sphere at a value of $10mm^4N^{-1}s^{-1}$. This was done by adding other cuboids above or below the cube so that the relative position of the sphere could be varied. Such a situation is also symmetrical; however, since the depth is being varied, the whole depth of the cube must be modelled and symmetric conditions can only be

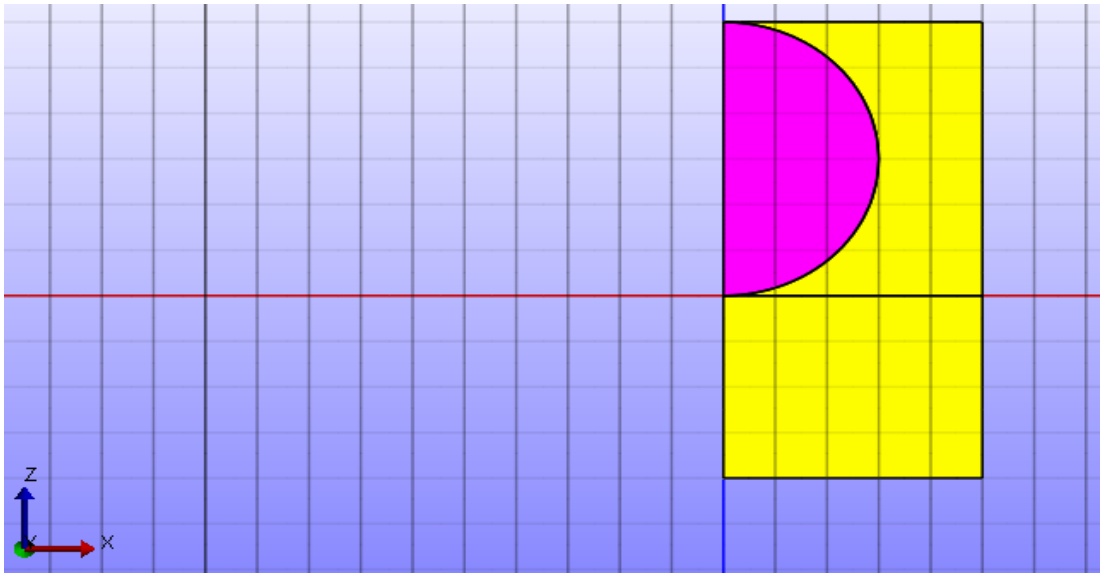


*Figure 11: Modelling the problem using symmetric conditions for $x/h \neq 0.5$*

applied to 1/4$^{th}$ of the actual problem. Thus in this case, the situation was modelled as shown in Figure 11.

All studies were carried out using a Finite Element software package consisting of PreView (for modelling the problem), FEBio (for solving the modelled problem), and PostView (for obtaining results and post-processing).

## Material model

A biphasic material model having 0.3% solid was used for both the sphere and the cube. The solid phase was set to have Neo-Hookean material properties with Poisson's ratio having a value of 0.125 for both geometries. The Young's Modulus was set to a value of $0.9642MPa$. for the cube, whilst for the sphere, the Young's Modulus was varied to represent different situations. The liquid phase was set to have a density of 1kg/mm$^3$ for both geometries. The hydraulic permeability was set to a value of $100mm^4N^{-1}s^{-1}$ for the cube, whilst it was also varied for the sphere in order to model different situations.

## Meshing

A fine mesh near the contact interface as shown in Figure 13 was used so that the interaction between the two substances could be appropriately modelled with a reduction in error. In situations like this, it is essential to show that the results are independent of the mesh density used. If a mesh that is not dense enough is used, the results might be inaccurate and will vary according to the mesh density used. This means that the problem would not be accurately modelled.

### Mesh Sensitivity Study

The ideal mesh size in this case was found by carrying out a mesh sensitivity study for the case where $x/h = 0.5, r/h = 0.3$ and with the sphere having material properties:

- $E = 96.42MPa$
- $k = 10mm^4N^{-1}S^{-1}$
- $r/h = 0.3$

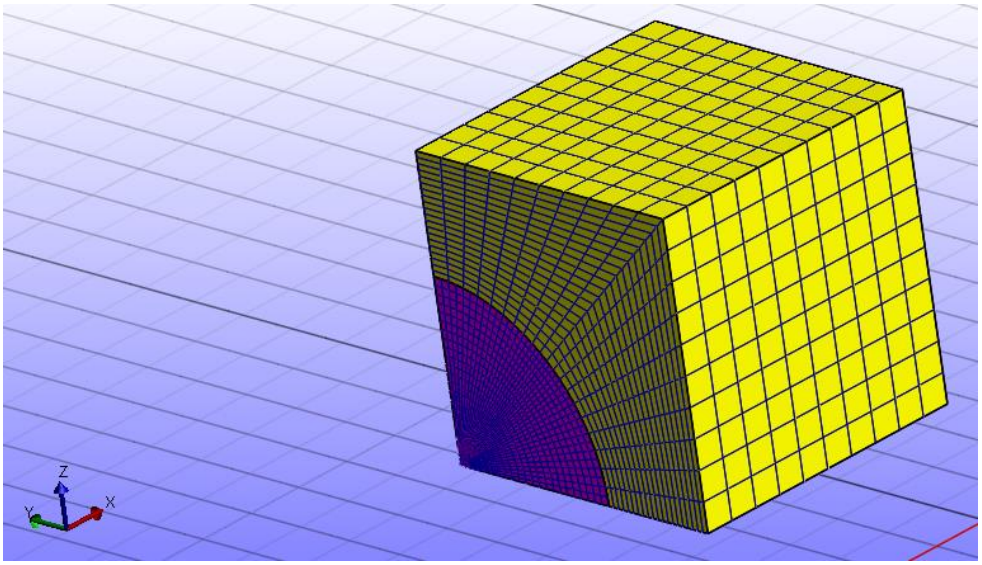


*Figure 13: The mesh useed with x/h=0.5*

In this study, meshes that represented the edges of the cube having 0.8div/mm, 1div/mm, 2div/mm, and 3div/mm were used, and the mesh sizes at the sphere and the interface were adjusted accordingly. The model was loaded in confined compression as further described below, and the variation of peak force with mesh density was plotted as shown in Figure 12. From this it could be observed that at lower mesh densities, the recorded force is higher than at higher densities. Also, at mesh densities larger than $2div/mm$ it can be noticed that the force recorded does not produce large variations and in fact it starts to converge to increase again as the mesh density becomes larger, however, it does this at a slower rate and we can assume that

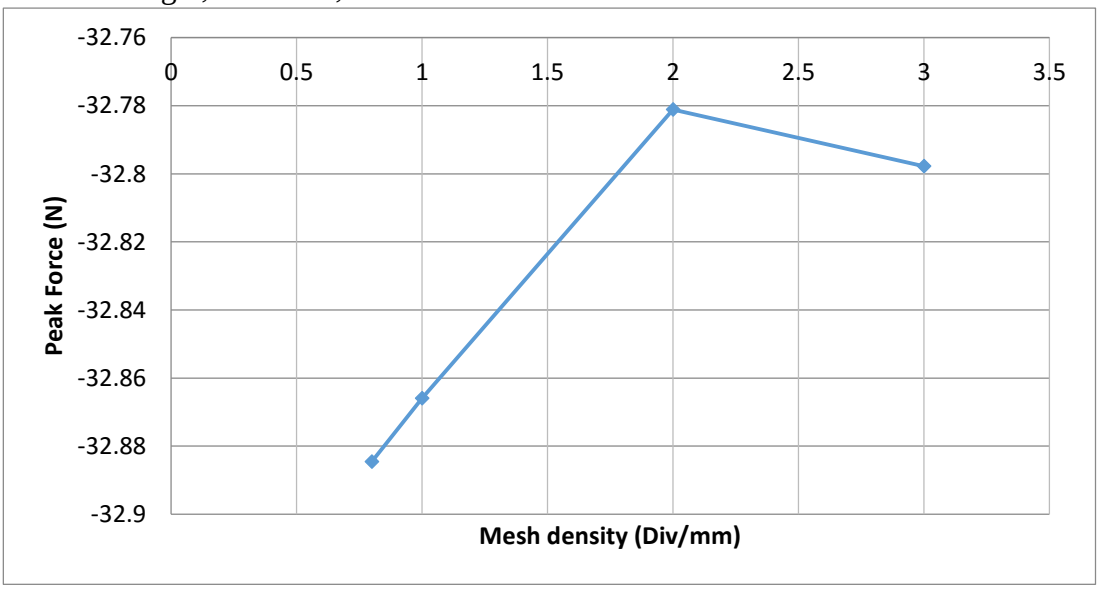


*Figure 12: Results obtained from the mesh sensitivity study*

eventually it starts converging to a fixed value that is not much different than the peak force observed with a mesh sensitivity of $2div/mm$ across the sides of the cube. For this reason it was decided that a mesh density of $2div/mm$ would be ideal in this study since it demands the least computational power and it still provides relatively accurately results.

## Boundary conditions

### $x/h = 0.5$

For the situation when $x/h = 0.5$, symmetric boundary conditions were applied by restricting movements of the lateral faces in the x- and y- directions and by restricting movement of the bottom face in the z-direction. A constraint that restricts pressure at the top surface to a magnitude of 0MPa was also included in order to model the porous

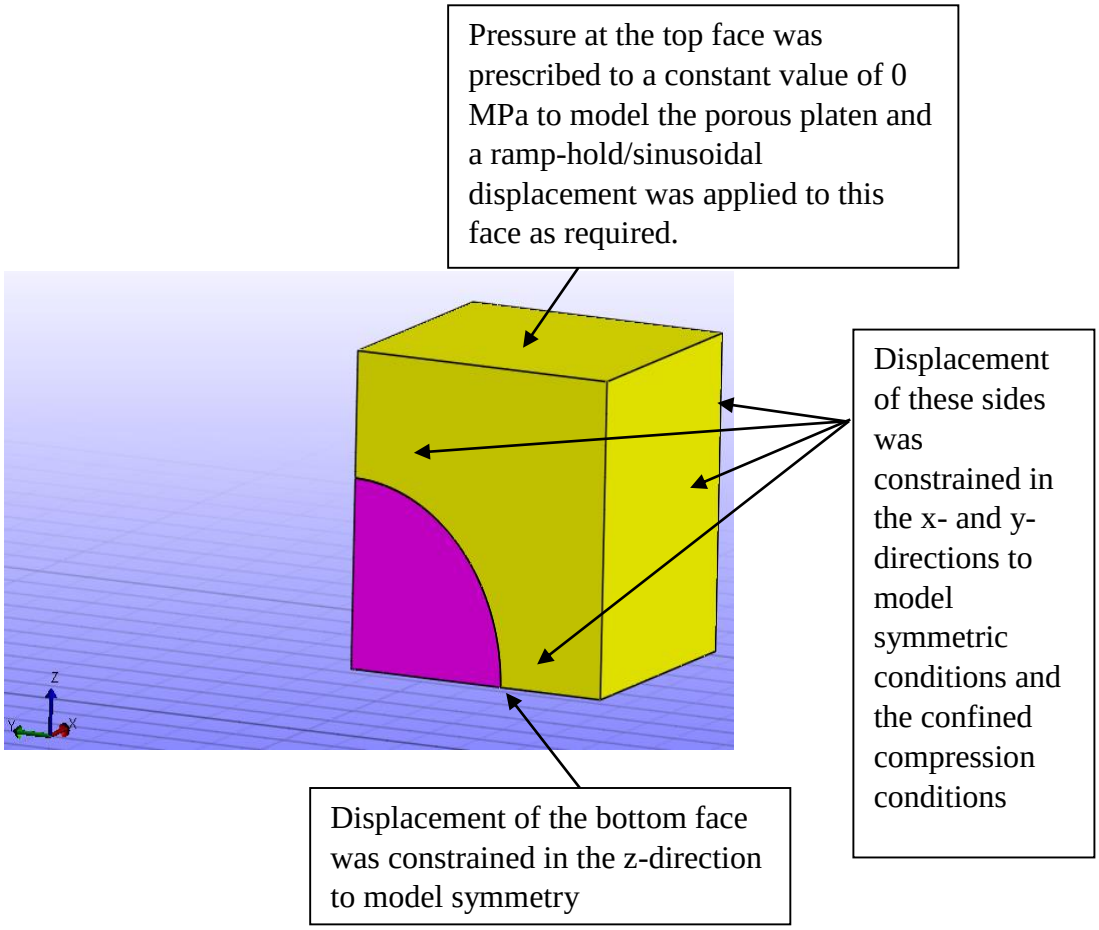


*Figure 14: Loads and Boundary conditions that were applied to the model with $x/h = 0.5$*

platen that compresses the hydrogel as shown in Figure 9. The final model had the boundary conditions shown in Figure 14.

### $x/h \neq 0.5$

For the situation when $x/h \neq 0.5$, symmetric boundary conditions were again applied by restricting movements of the lateral faces in the x- and y- directions. The bottom surface was also restricted in the z-direction together with a prescribed pressure of 0 MPa so as to model the fixed porous platen at the bottom. A constraint that restricts pressure at the top surface to a magnitude of 0 MPa was also included again in order to model the porous platen that compresses the hydrogel as shown in Figure 15.

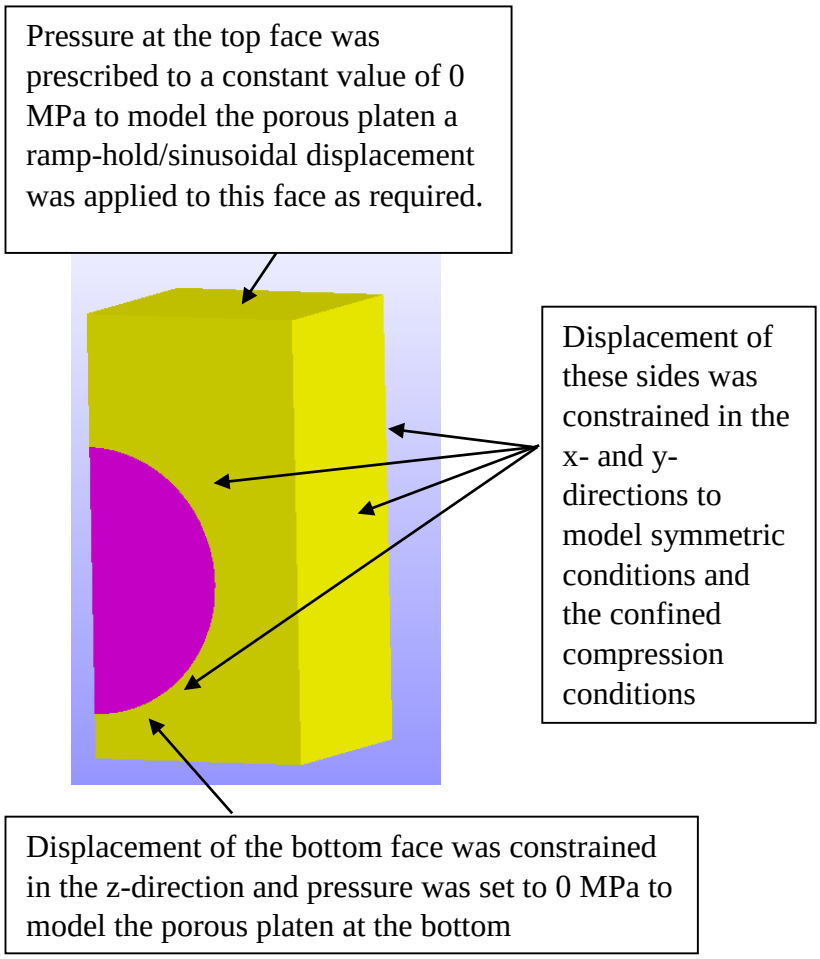


*Figure 15: Loads and Boundary conditions that were applied to the model with $x/h \neq 0.5$*

## Contact

A tied biphasic interface was used at the sphere-cube interface and at the cube-cube interface in order to modify the depth of the sphere in the hydrogel in the case when $x/h \neq 0.5$. This was done because such an interface allows the fluid phase to flow between different geometries across the boundary. When using this type of contact, the surface of the sphere was set as the "master" whilst the surface of the cube touching the sphere was set as the "slave". The reason for this is that it is the effect of the surface of the sphere that affects the response of the cube. It is important to mention that the contact was validated prior to use. Validation was carried out by first modelling a cube of hydrogel only, and then modelling the sphere inside the hydrogel with both having the same material properties and with $r/h = 0.3$. Contact settings were also modified by using the following settings:

- Augmented tolerance=0.02
- Auto-penalty = Yes
- Two-pass = Yes
- All other settings were set at the default values

These settings were changed because the default values were providing an intersection of the two geometries that was too large (Figure 16) and this could have resulted in inaccuracies. After changing these settings, the intersection between the two geometries was reduced as can be observed in Figure 17.

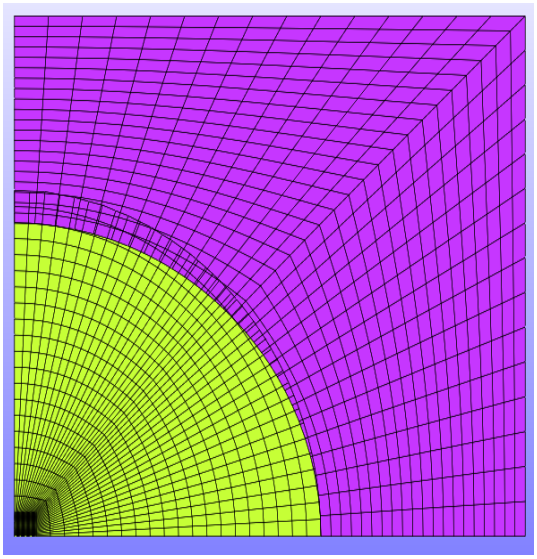

*Figure 16: The model loaded under ramp-hold conditions without optimised contact settings*

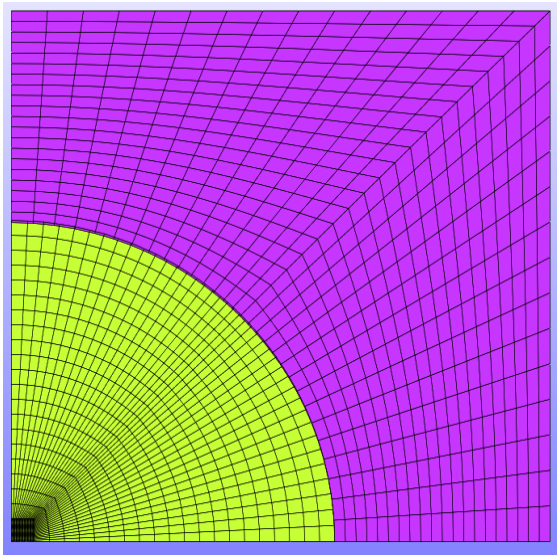

*Figure 17: The model loaded under ramp-hold conditions with optimised contact settings*

The contact was validated by analysing the force exerted by the platen on the hydrogel and the variation of fluid flux with depth of the cube for the three different conditions subjected to a ramp-hold strain:

- Hydrogel only.
- Hydrogel with the sphere having the same material properties without optimising contact.
- Hydrogel with the sphere having the same material properties with optimised contact.

The results obtained for the variation of force with time and for the maximum fluid flux with depth are shown in Figure 18 and Figure 19.

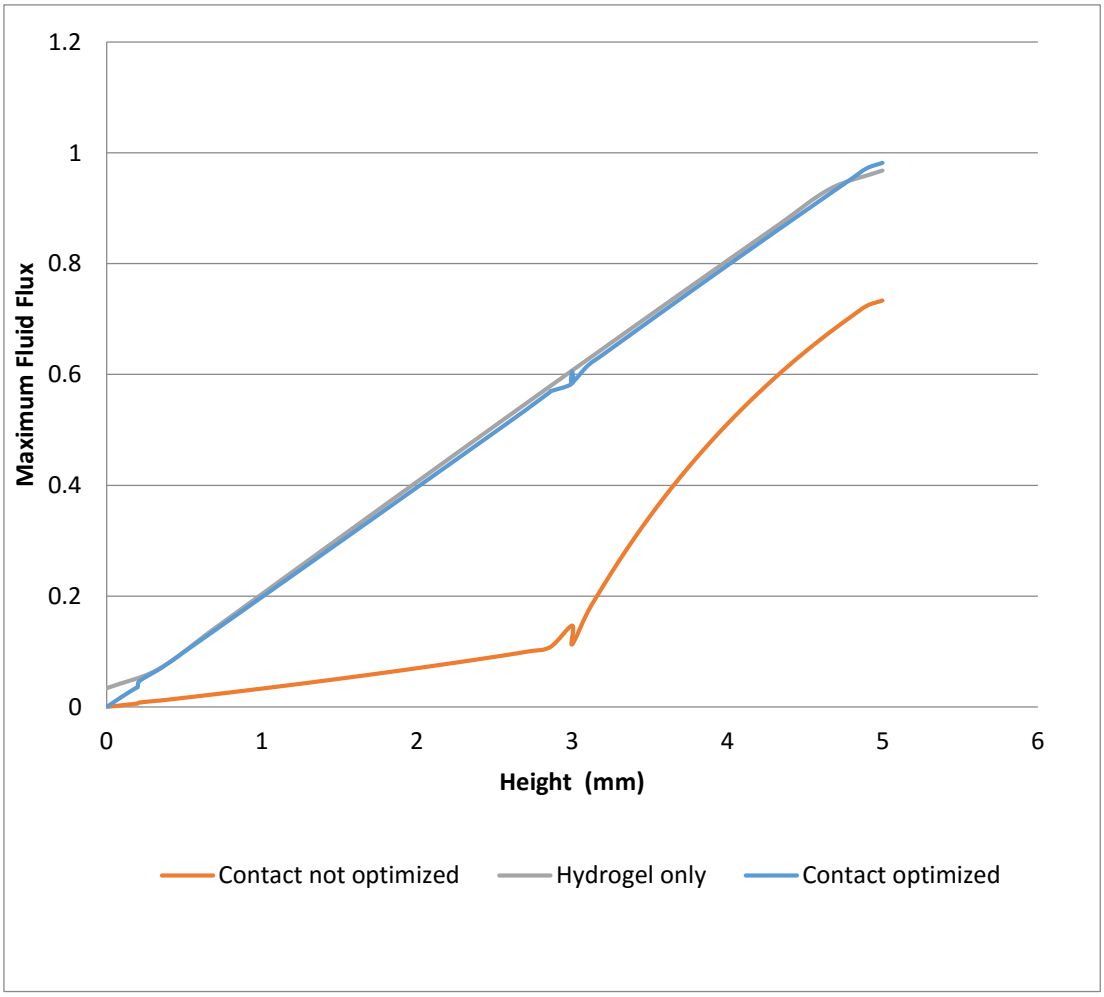


*Figure 18: The variation of fluid flux with height*

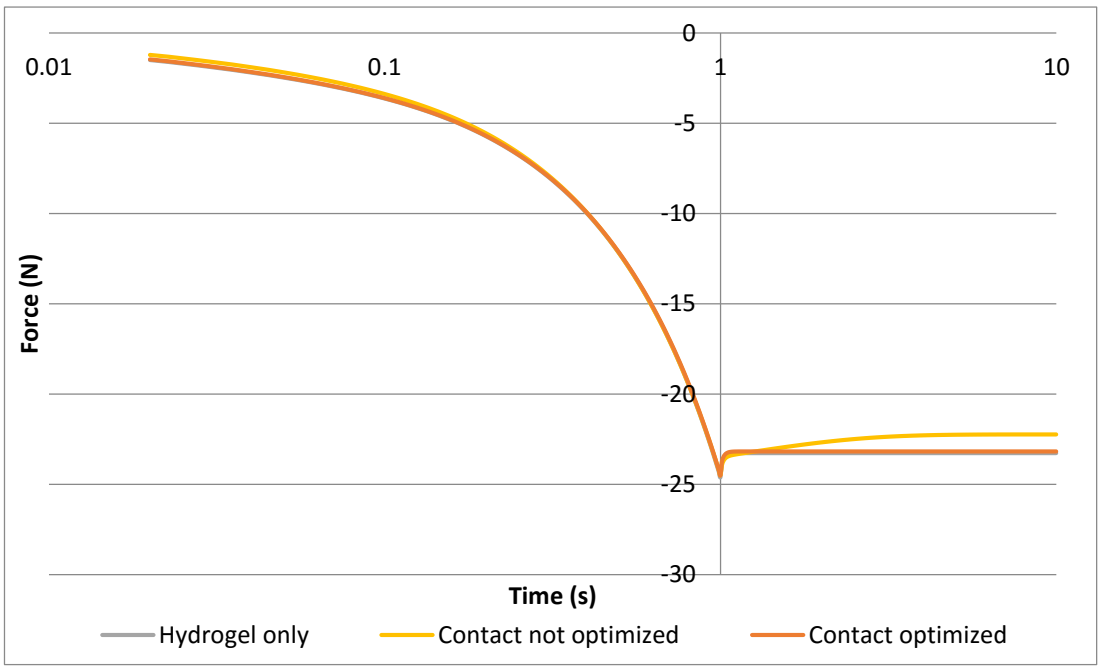


*Figure 19: The force-time response of the model when validating the contact*

As can be observed from Figure 19, the force response in each case is very similar; however, there is a small difference in the equilibrium response when the contact is not optimised and there is a large intersection between the meshes of the sphere and the cube respectively. One must also note that the representation of fluid flux with respect to height is not correctly mapped when the contact is not optimised and the maximum fluid flux was also observed to occur at a different time in this case. On the other hand, when it is optimised using the previously mentioned parameters, the response is much closer to the control response (hydrogel only) and the peak fluid flux occurs at the same time as when only hydrogel is modelled. This shows that the contact is working appropriately when it is optimised according to the already mentioned settings and that it can be used to model the situation in an accurate manner.

## Loads

All models were loaded in confined compression by applying displacements to the porous platen at the top platen, in two different conditions:

- Ramp-hold displacement to the top surface of the cube reaching a maximum compressive strain of 20% in 1 second. This was obtained by applying a displacement to the top face as shown in Figure 20 in the case when $x/h =$

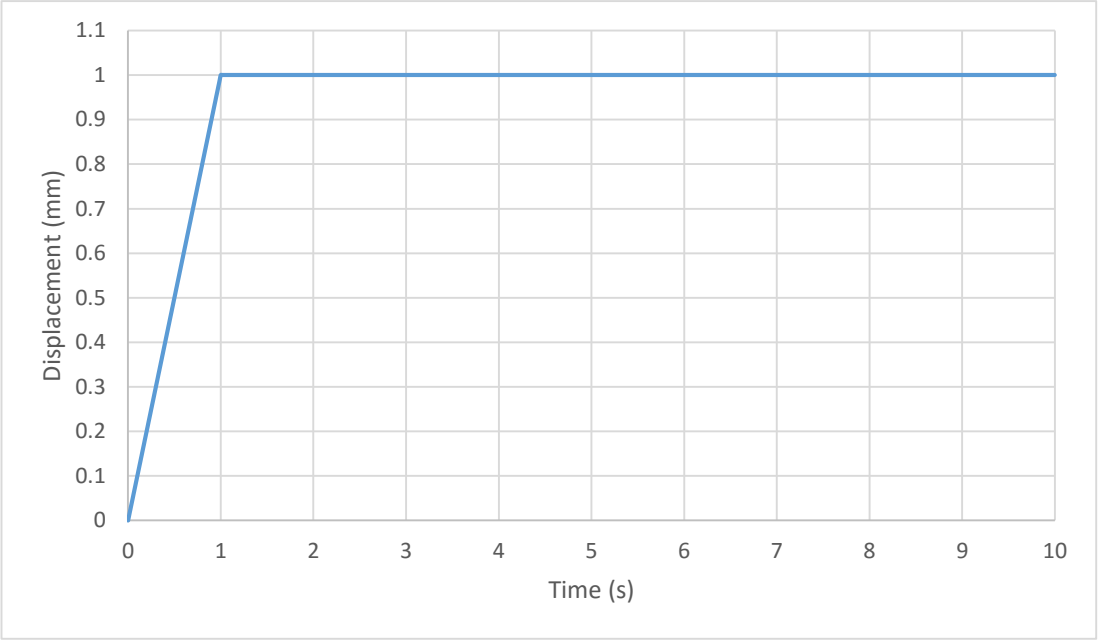


*Figure 20: The ramp-hold displacement applied to the top porous platen*

0.5. Due to the boundary conditions that were applied, a displacement of 1mm will give a strain of 20% since only half the depth of the cube is modelled. In the case when $x/h \neq 0.5$, a displacement of 2mm had to be used in order to obtain a strain of 20% since there are no symmetric boundary conditions along the depth of the cube.

- Sinusoidal displacement applying a strain according to the displacement equation: $-0.5 + 0.5\sin\left(2\pi Ft + \frac{\pi}{2}\right)$. This results in a sinusoidal strain oscillating at a frequency of $F$Hz between magnitudes of 0% up to a maximum of $20\%$ strain in the case when $x/h = 0.5.$. This was obtained by applying a displacement to the top face as shown in Figure 20 in the case when $x/h = 0.5$. Due to the boundary conditions that were applied, a displacement of 1mm will give a strain of 20% since only half the depth of the cube is modelled. In the case when $x/h \neq 0.5$, a displacement of 2mm had to be used in order to obtain a strain of 20% since there are no symmetric boundary conditions along the depth of the cube. When using a sinusoidal load, the frequency was also varied between $1Hz$ and $10Hz$ in increments of $2Hz$ in order to examine the response under different frequencies. This resulted in a sinusoidal compression of the model as indicated by Figure 21.

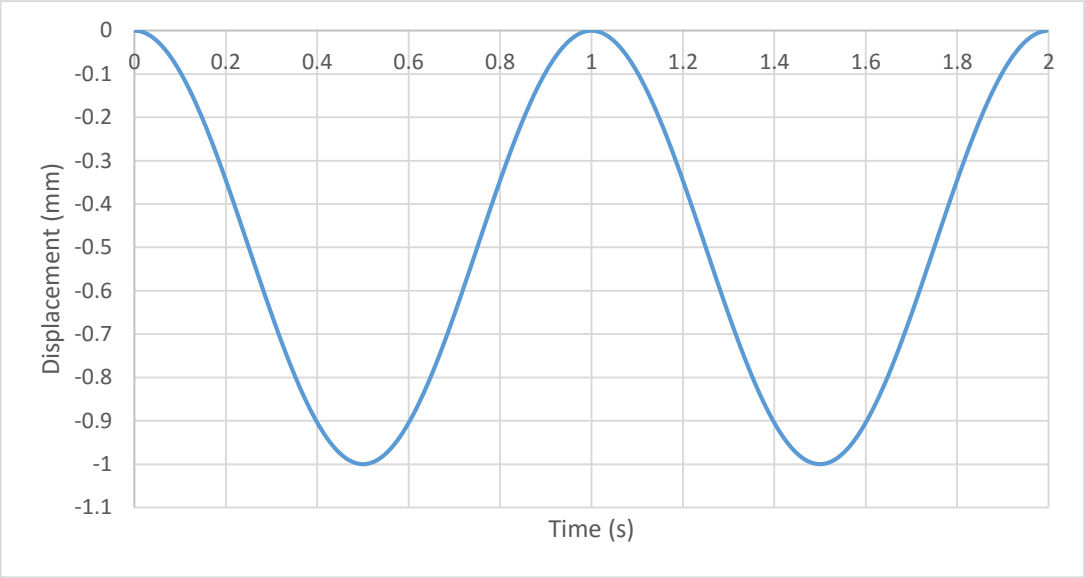


*Figure 21: The sinusoidal displacement applied to the top porous platen with $F = 1Hz$*

## Solver settings

### Ramp-hold loading

When applying a ramp-hold strain to the material, a biphasic solver was used in FEBio. The simulation was subdivided into two steps. The first step consisted of the first 2 seconds of the simulation (where the response of the material is more unstable). This step consisted of 200 time-steps having a maximum step size of 0.01 seconds. This ensured that enough steps were used in order to accurately obtain the dynamic response of the material. The second step consisted of the last 8 seconds of the simulation (where the material is approaching an equilibrium condition). In this case, 80 time-steps were used with a maximum step size of 0.1 because the response of the material is not expected to be highly dynamic, and thus, a lower amount of steps can be used in order to model the response without demanding excessive computational time and power.

### Sinusoidal loading

When applying a sinusoidal strain to the material, the biphasic solver was set to have a maximum of 200 time-steps with a maximum step size of 0.01s. This means that the duration of the study lasts a total of 2 seconds. In this case, a large number of time-steps is also required since the response of the material is expected to be highly dynamic and equilibrium conditions will surely not be reached.

## Obtaining results

The files created in PreView using the previously described conditions and settings were then exported so that they could be run using FEBio. A batch file was created to run all the simulations continuously. FEBio automatically generates an output plot file that can be opened with PostView. These files were then analysed using the post-processing software (PostView). The force exerted by the platen onto the material was obtained in PostView. This was done by selecting all the elements at the top surface and integrating the z-stress at these elements with respect to area. This force was analysed because it is one of the easiest parameters that can be recorded in experimental study and thus it facilitates the comparison between theoretical and experimental results if such experiments are carried out in future studies.

## Results

### Ramp-Hold strain

#### Varying the parameter $r/h$

The response of the system with variation of $r/h$ can be observed in Figure 22 to Figure 23 below. One may note that as $r/h$ increases, force applied by the platen on the material increases. At very small values of $r/h$ (0.05 and 0.1) there is negligible difference in the response for different values of $r/h$; however, as $r/h$ increases and exceeds a value of 0.2, the rate of change of force (both peak and equilibrium) increases and results in a larger force response being observed such that the response becomes dependent on the size of the sphere inside the cube as shown in Figure 24 and Figure 23.

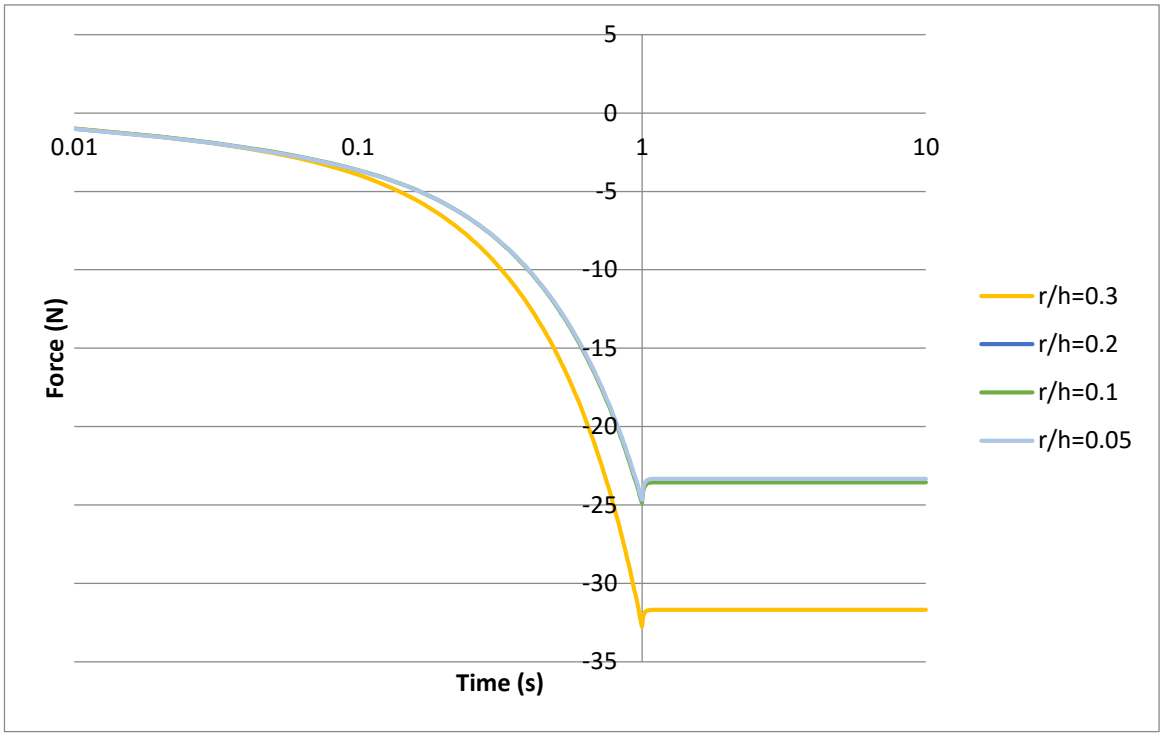


*Figure 22: The effect of r/h on the time response of the material*

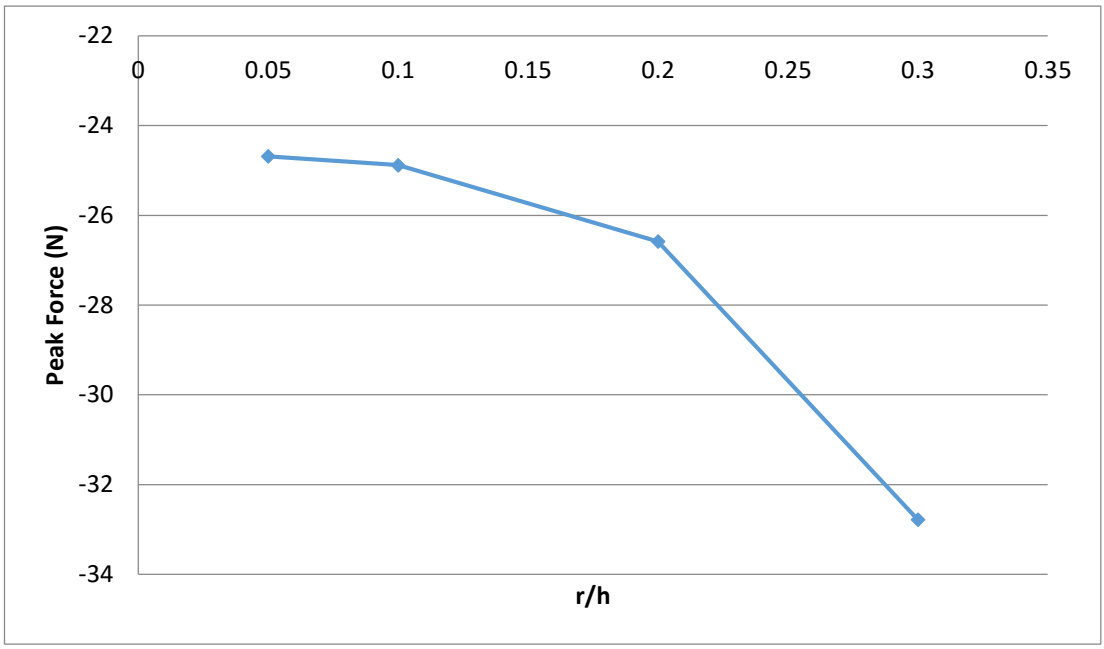


*Figure 24: The variation of peak force with respect to r/h*

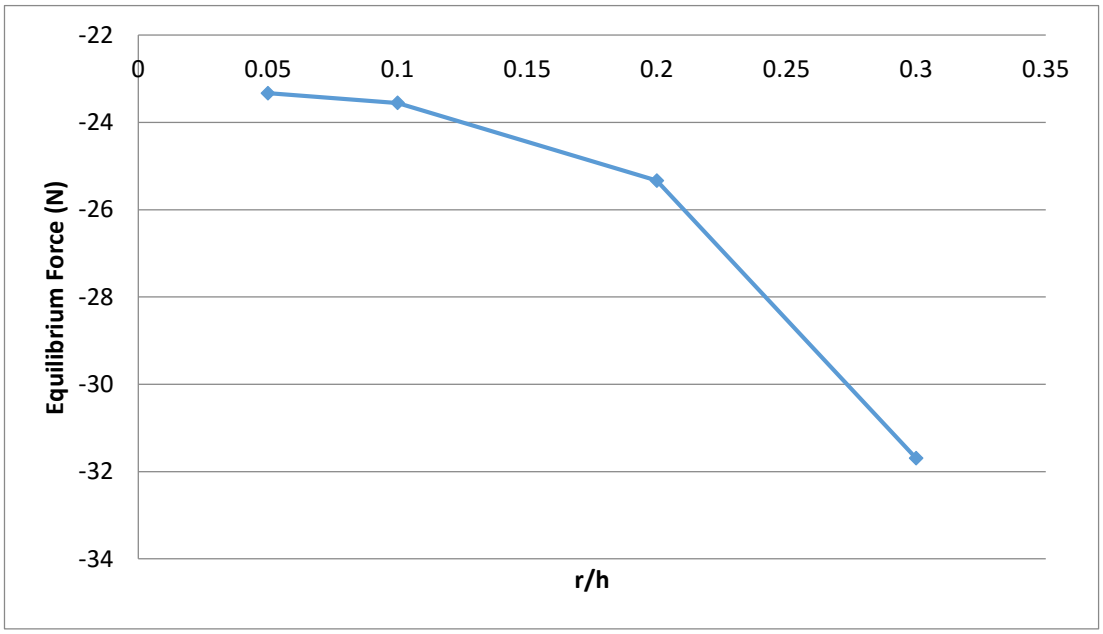


*Figure 23: The variation of equilibrium force with respect to r/h*

## Varying the Hydraulic Permeability

The response of the system as the hydraulic permeability of the sphere is decreased can be observed in Figure 25 to Figure 29 below. One may note that as the hydraulic permeability decreases, both the peak and equilibrium forces increase. The responses for $k = 100mm^4N^{-1}s^{-1}$ and $k = 10mm^4N^{-1}s^{-1}$, are very similar and differences

are barely detectable however if there is a further reduction in permeability, the change in response becomes very easy to detect since there is a larger difference in force magnitude. One must also note that the hydraulic permeability affects the response to ahigher degree at low values of Young's Modulus. As the stiffness is increased, the response becomes less dependent on the hydraulic permeability.

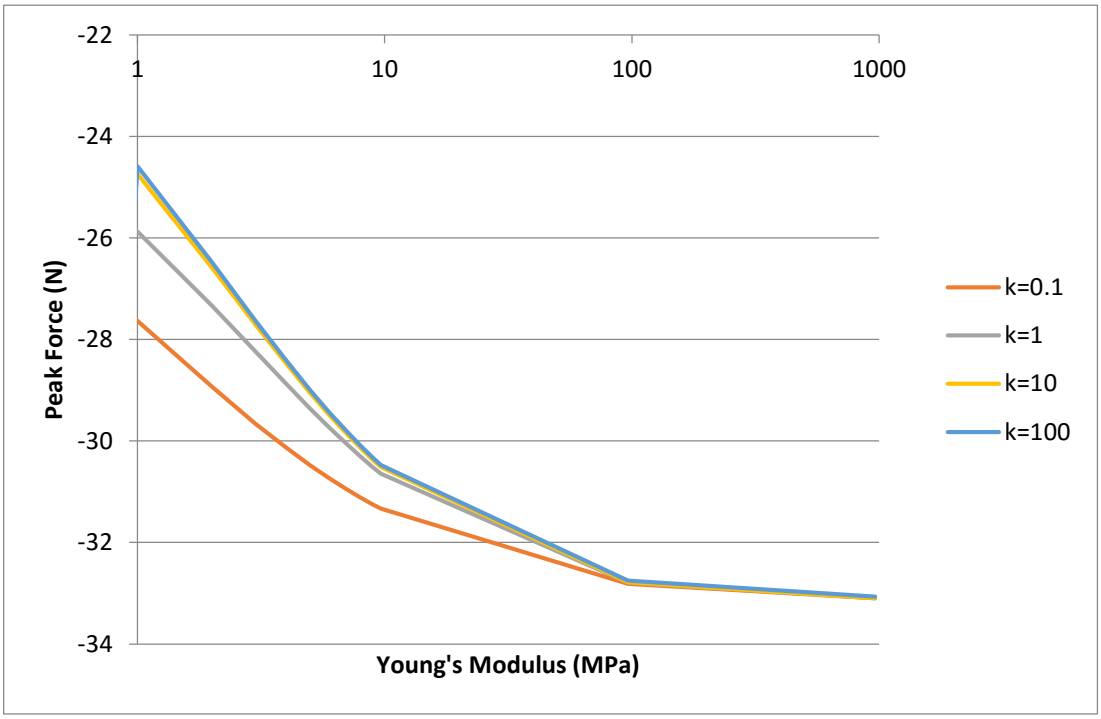


*Figure 25: A plot of Peak Force vs. Young's Modulus for different values of Hydraulic Permeability*

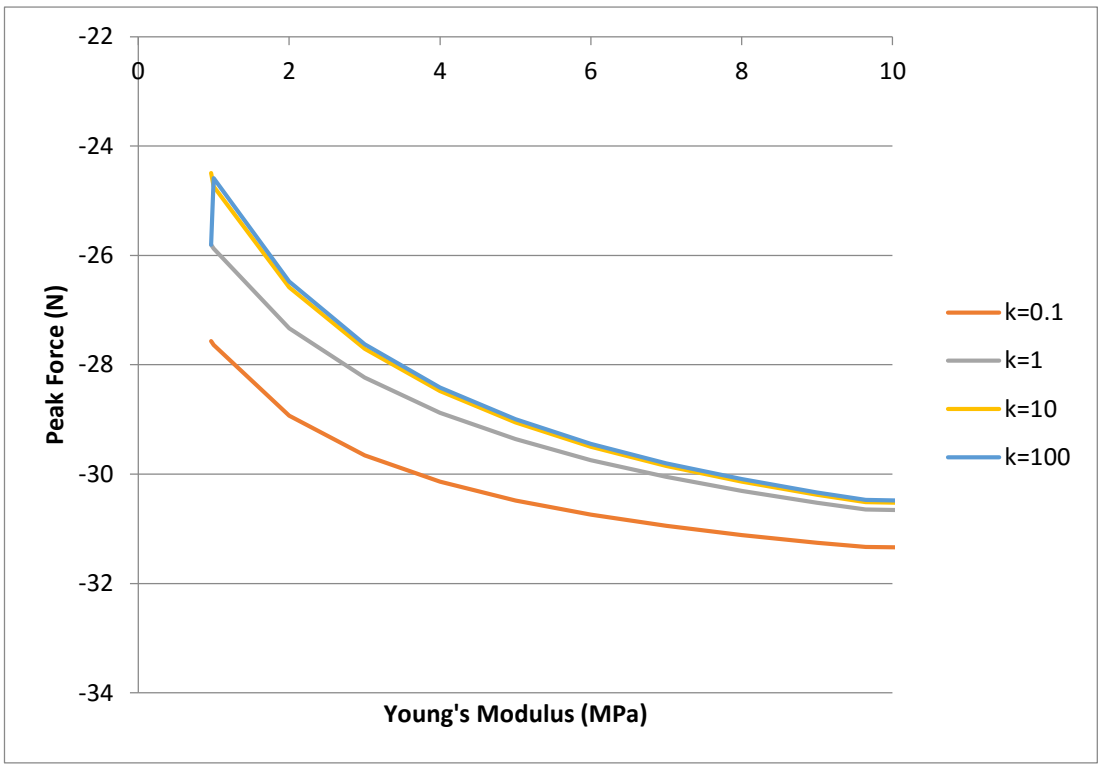


*Figure 27: A plot of Peak Force vs. Young's Modulus for different values of Hydraulic Permeability (magnified at low values of Young's Modulus)*

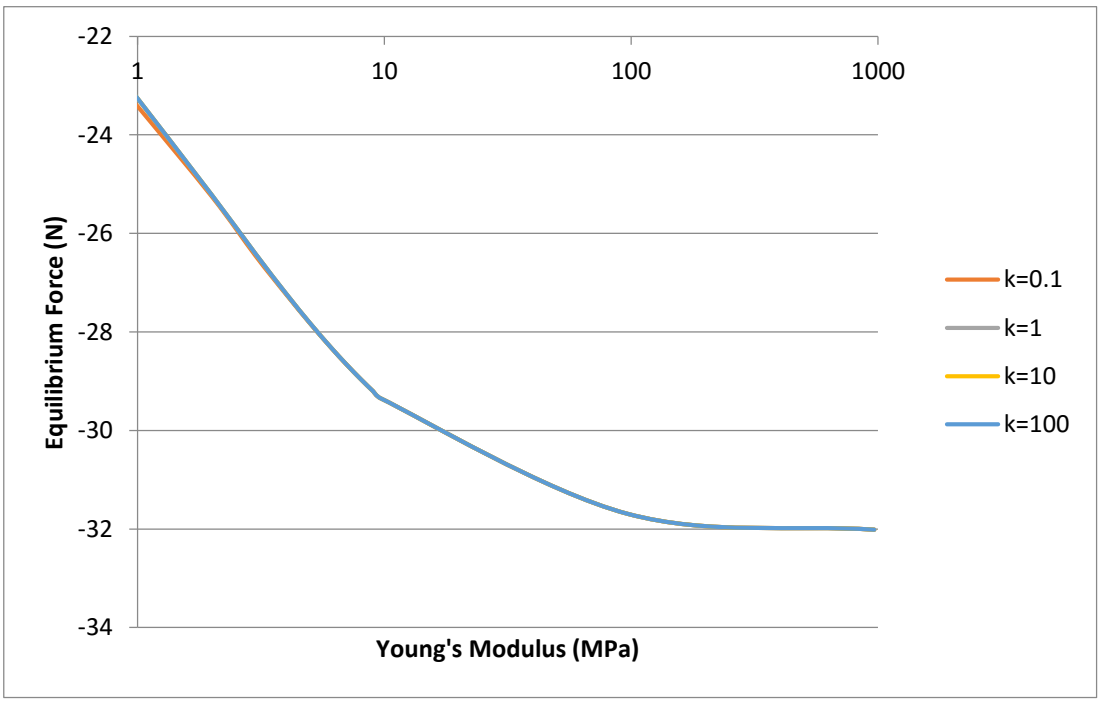


*Figure 26: A plot of Equilibrium Force vs. Young's Modulus for different values of Hydraulic Permeability*

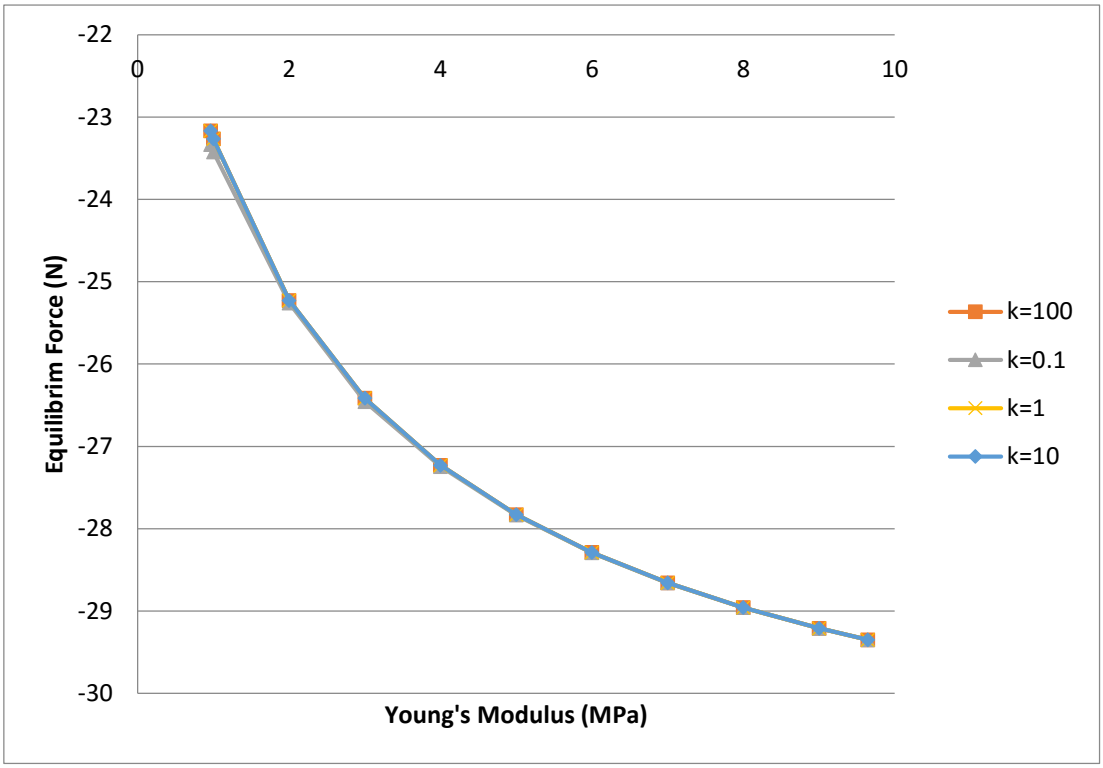


*Figure 29: A plot of Equilibrium Force vs. Young's Modulus for different values of Hydraulic Permeability (magnified at low values of Young's Modulus)*

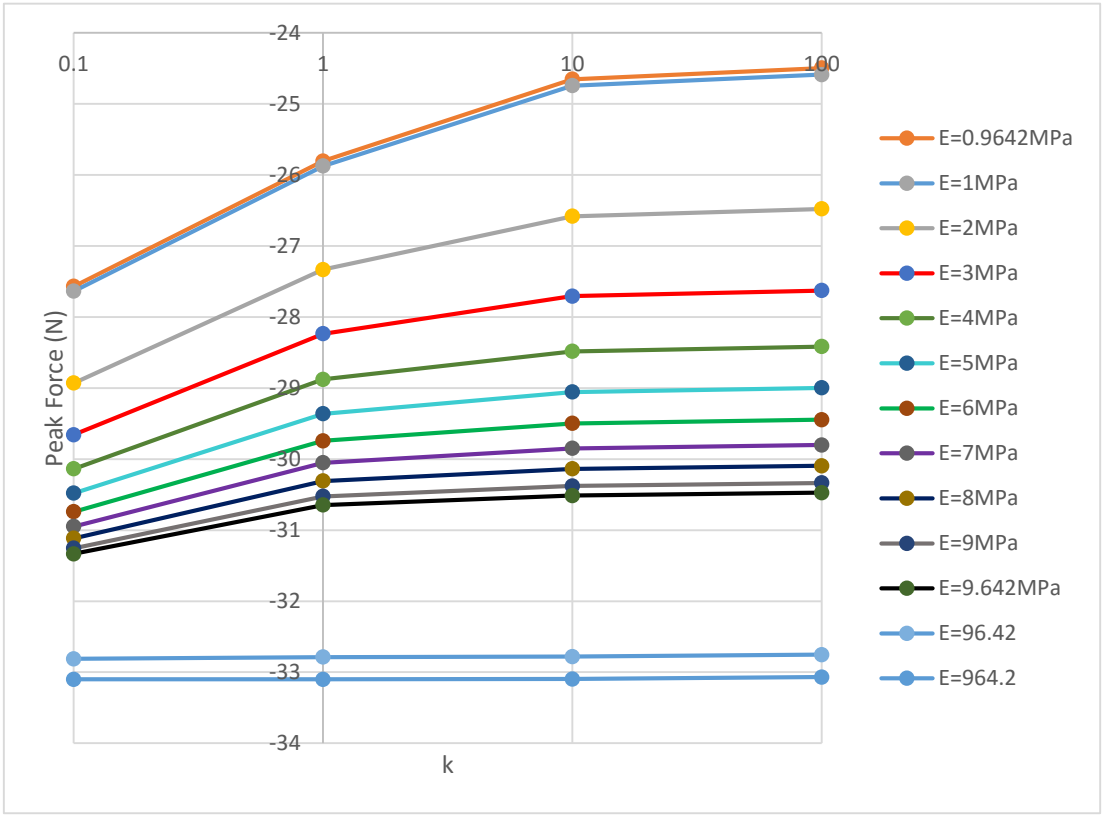


*Figure 28: A plot of Peak Force vs. Hydraulic Permeability for different values of Young's Modulus*

### Varying the Young's Modulus

The response of the system as the Young's Modulus of the sphere is increased can be observed in Figure 28 and Figure 30. One may note that as the Young's Modulus increases the peak and equilibrium forces increase. As one can observe, at $E < 9.642MPa$, the hydraulic permeability affects the peak force; however, as the Young's Modulus is further increased, the peak force becomes more dependent on the Young's Modulus and hydraulic permeability does not affect the response as much. The same effect can be observed for the equilibrium force; however in this case, the hydraulic permeability affects the response only when $E < 3MPa$. A hydraulic permeability of $10mm^4N^{-1}s^{-1}$ is also observed to decrease both the equilibrium force and the peak force response.

### Time response for Varying Material properties ($k$ and $E$)

From Figure 31, one may note that the time response for $k = 0.1$ at low values of Young's Modulus ($0.9642MPa < E < 2MPa$) is different from that at higher values of Young's Modulus. When considering the same time period, an equilibrium state

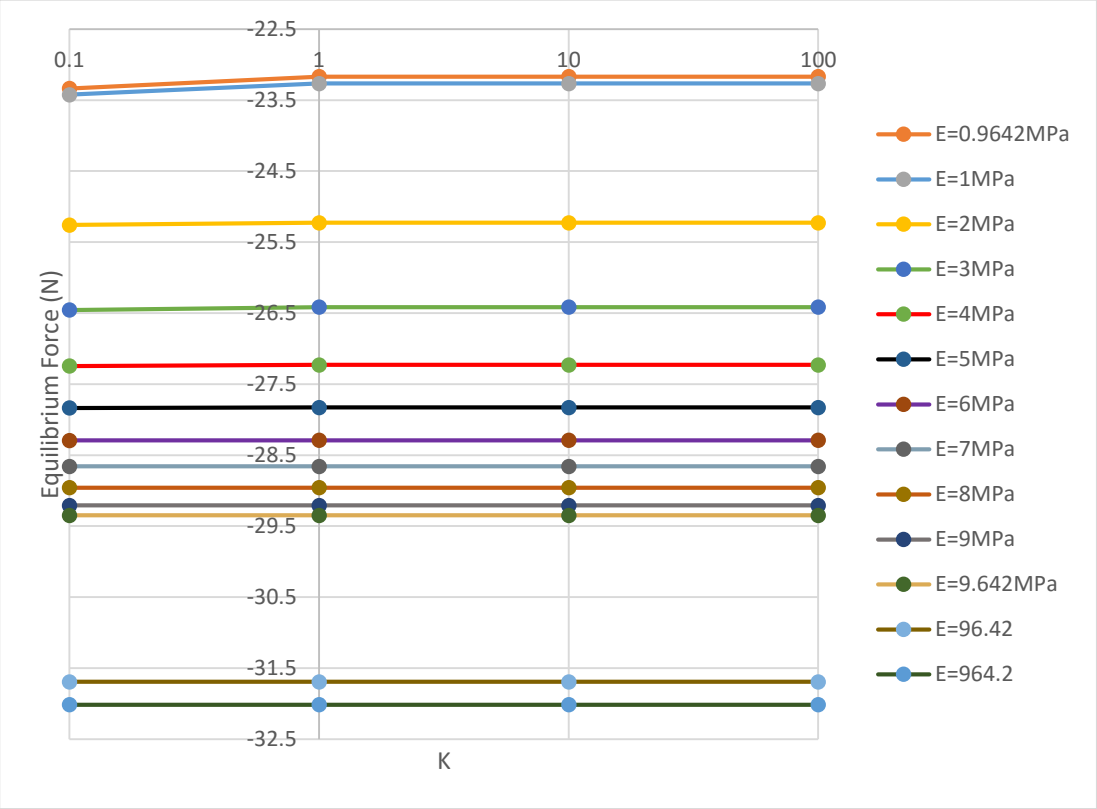


*Figure 30: A plot of Equilibrium Force vs. Hydraulic Permeability for different values of Young's Modulus*

could not be reached in such cases. For this reason, another four seconds of simulation were added to these cases and the response obtained from these may be observed in Figure 32 such that an equilibrium state could be reached. In all other cases, the response was also very similar and the only differences observed were only in the peak force reached and in the equilibrium. The raw data for these responses may be found in the electronic appendix attached to this project.

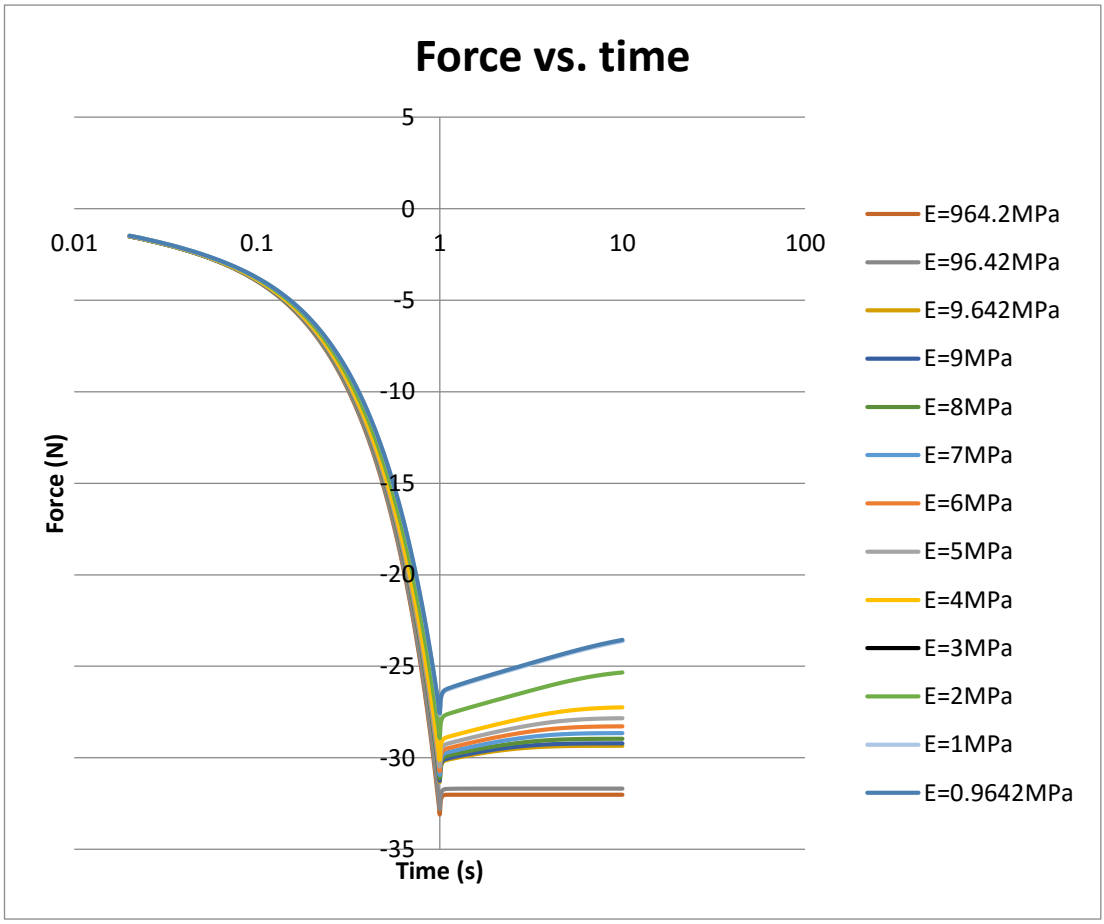


*Figure 31: Time response for k=0.1mm$^4$N$^{-1}$s$^{-1}$ without added time for equilibrium conditions*

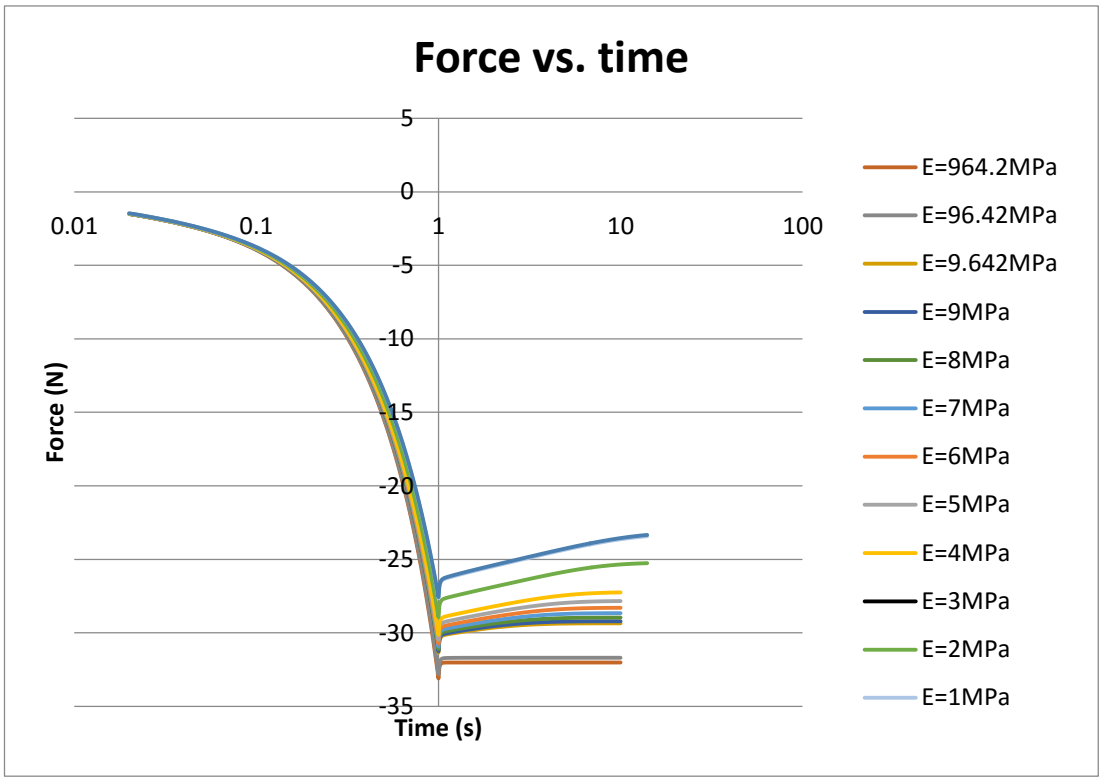


*Figure 32: Time response for k=0.1mm$^4$N$^{-1}$s$^{-1}$ with added time for equilibrium conditions*

### Varying the parameter $x/h$

The response of the system with variation of $x/h$ can be observed in Figure 34 to Figure 35 below. One may note that as $x/h$ increases, both the peak and equilibrium forces increase. When the sphere is located relatively close to the centre of the cube ($0.4 < x/h < 0.6$), the difference in time response is very similar; however, as the sphere gets closer to the edges of the cube, the peak/equilibrium force increases. One may note that the increase in force is also larger when the sphere is shifted towards the bottom side ($x/h = 0.7$) of the cube rather than towards the top side ($x/h = 0.3$).

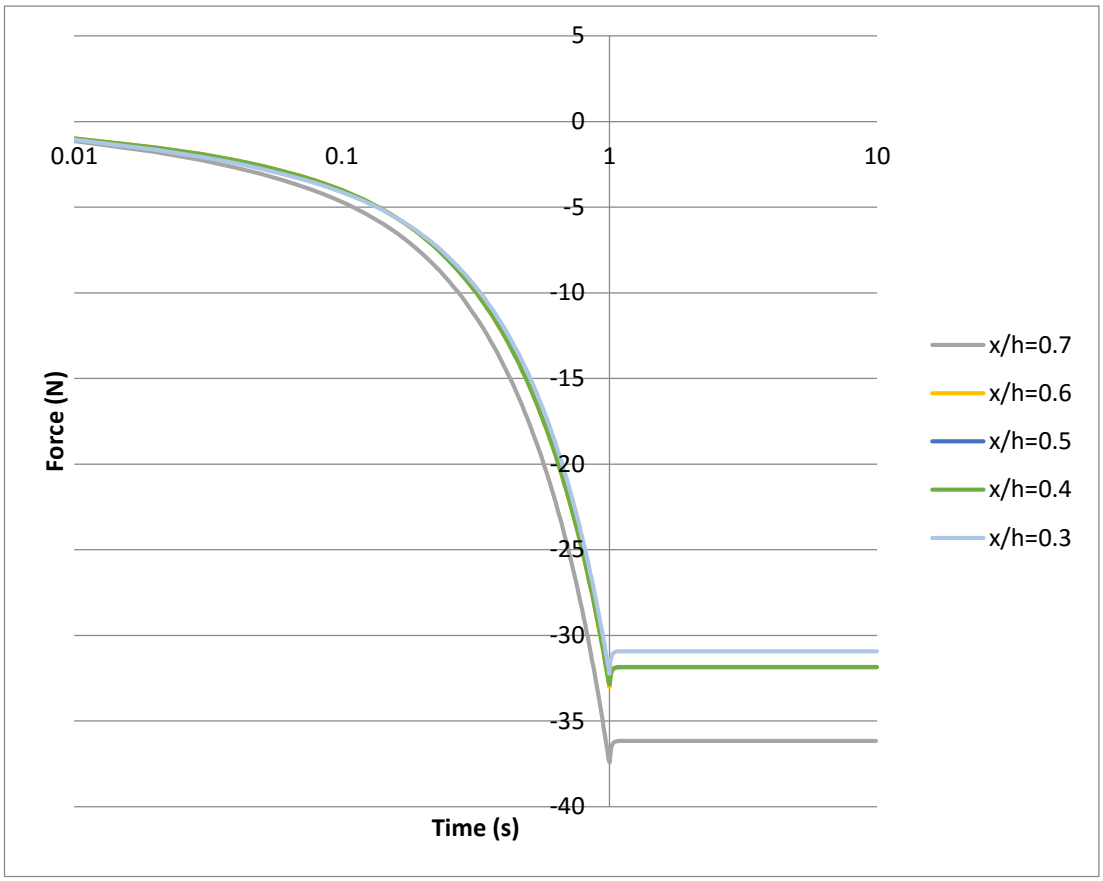


*Figure 34: The effect of x/h on the time response*

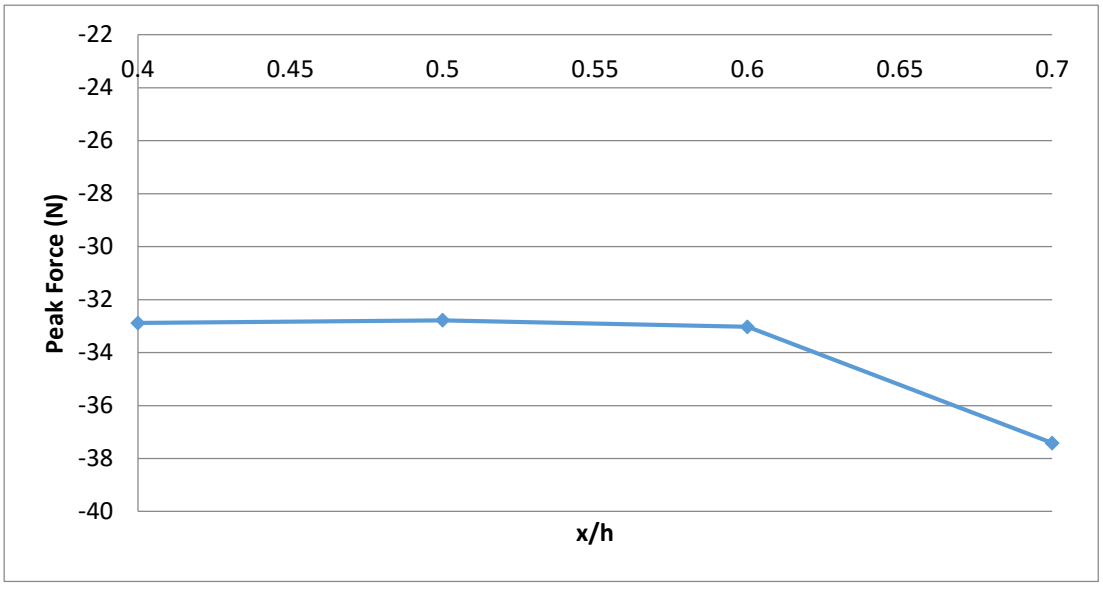


*Figure 33: A plot of Peak Force vs. x/h*

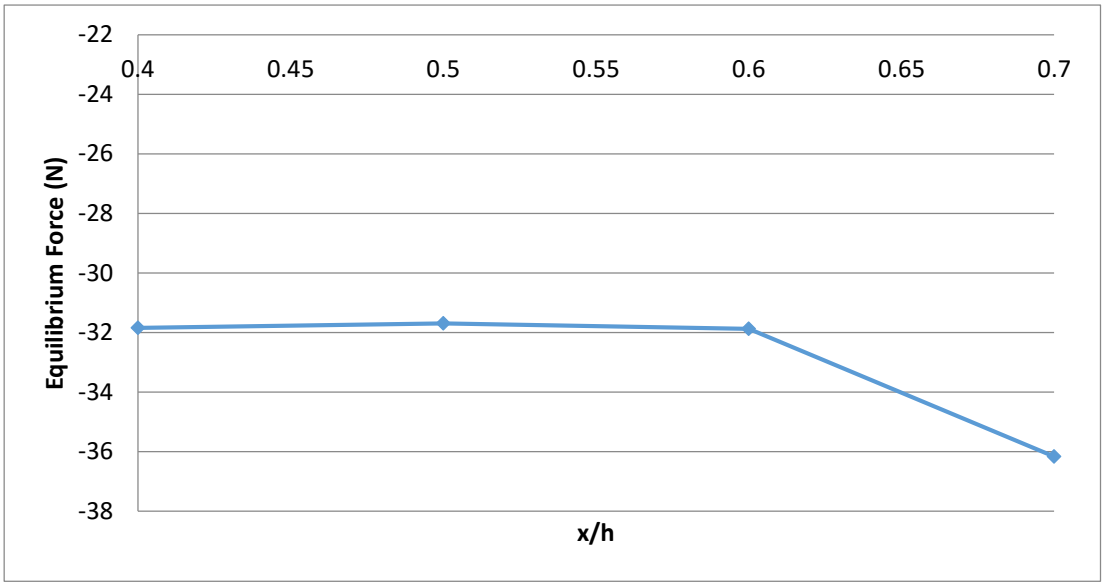


*Figure 35: A plot of Equilibrium Force vs. x/h*

## Sinusoidal strain

### Varying the parameter $r/h$

The effect that $r/h$ has on the observed maximum force magnitude can be observed in Figure 36 below. One may note that as $r/h$ increases, the peak force exerted by the platen on the material also increases. At very small values of $r/h$ (0.05 and 0.1) the gradient of the graph is much less than other values, which indicates that for very small values of $r/h$ a change in response might be difficult to detect using experimental techniques. A larger difference in response can be observed as $r/h$ exceeds a value of 0.1 and this can be even more easily detected as $r/h$ approaches even larger values.

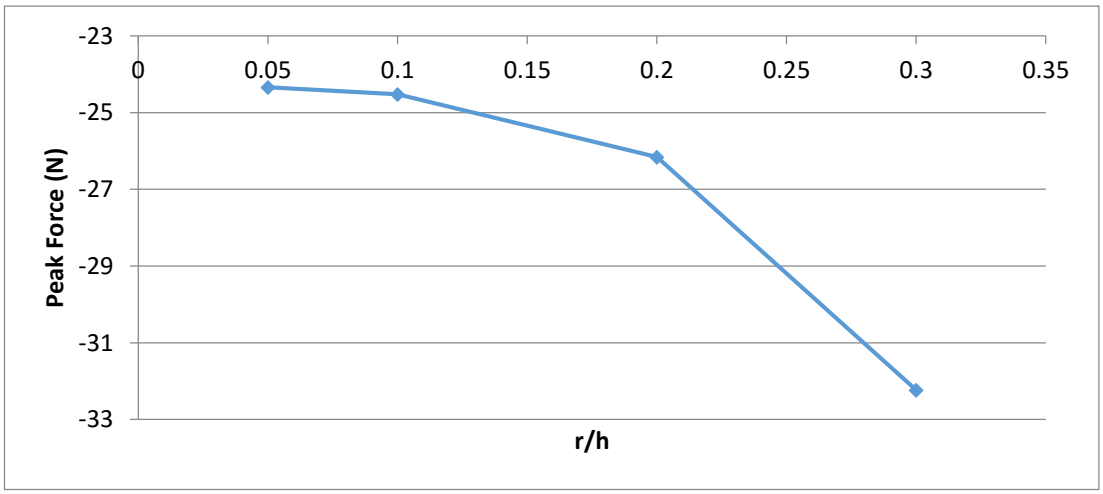


*Figure 36: The effect of r/h on the peak force magnitude*

A change in $r/h$ also contributes to a different response with respect to the phase lag observed ($\tan\delta$), which can be seen in Figure 37.

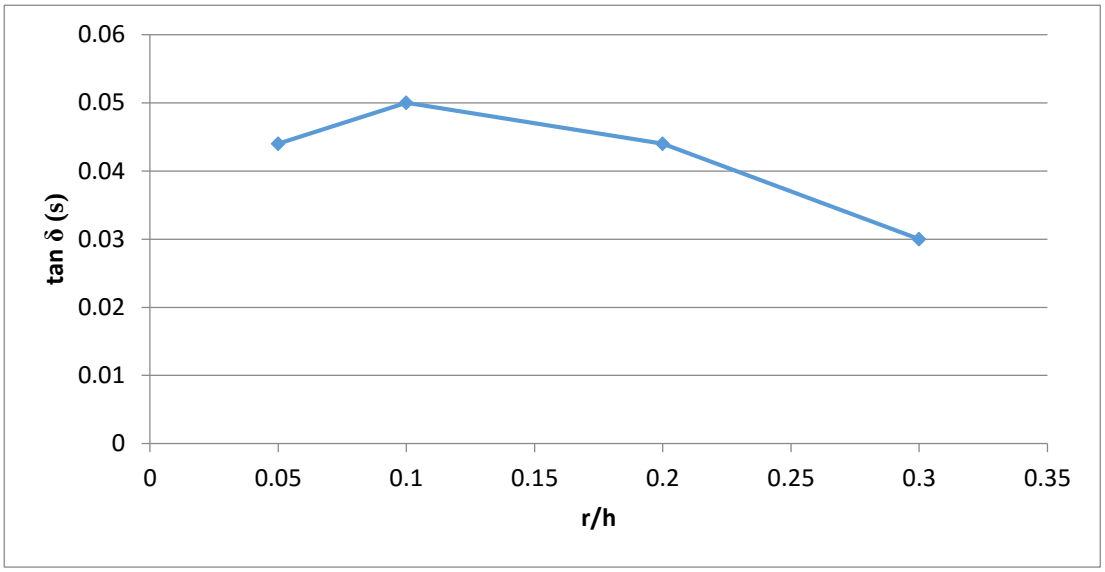


*Figure 37: The effect of r/h on phase lag*

## Varying the Hydraulic Permeability

The response of the system as the hydraulic permeability is decreased can be observed

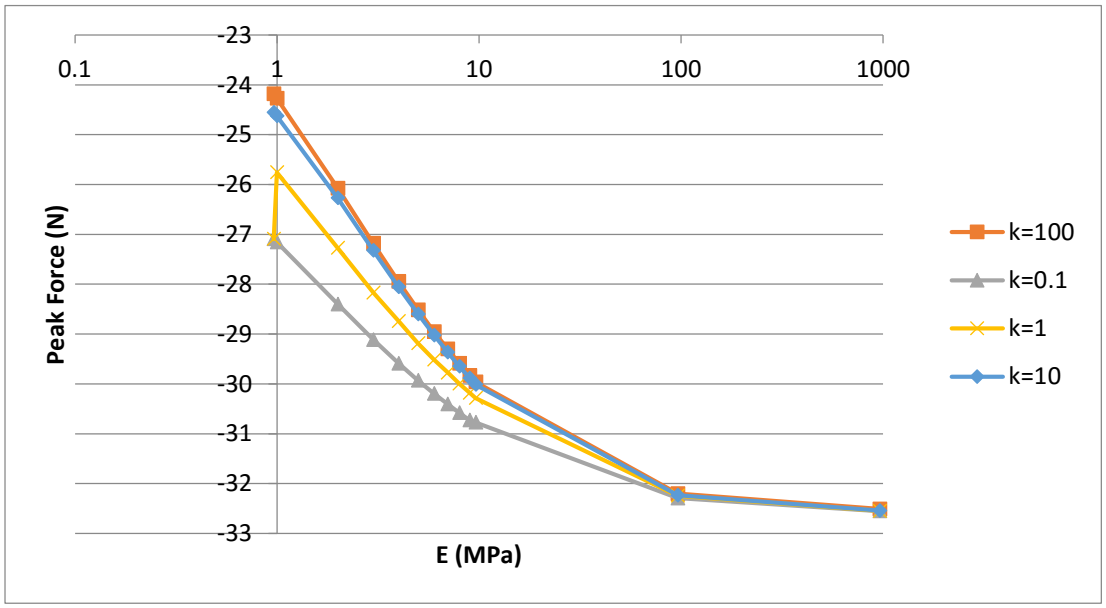


*Figure 38: The effect of hydraulic permeability on peak force*

in Figure 38. One may note that as the hydraulic permeability is decreased, the force magnitude applied by the platen on the material increases. Figure 39 also illustrates

the effect that hydraulic permeability has on the phase change; however there appears to be no obvious relationship in this case.

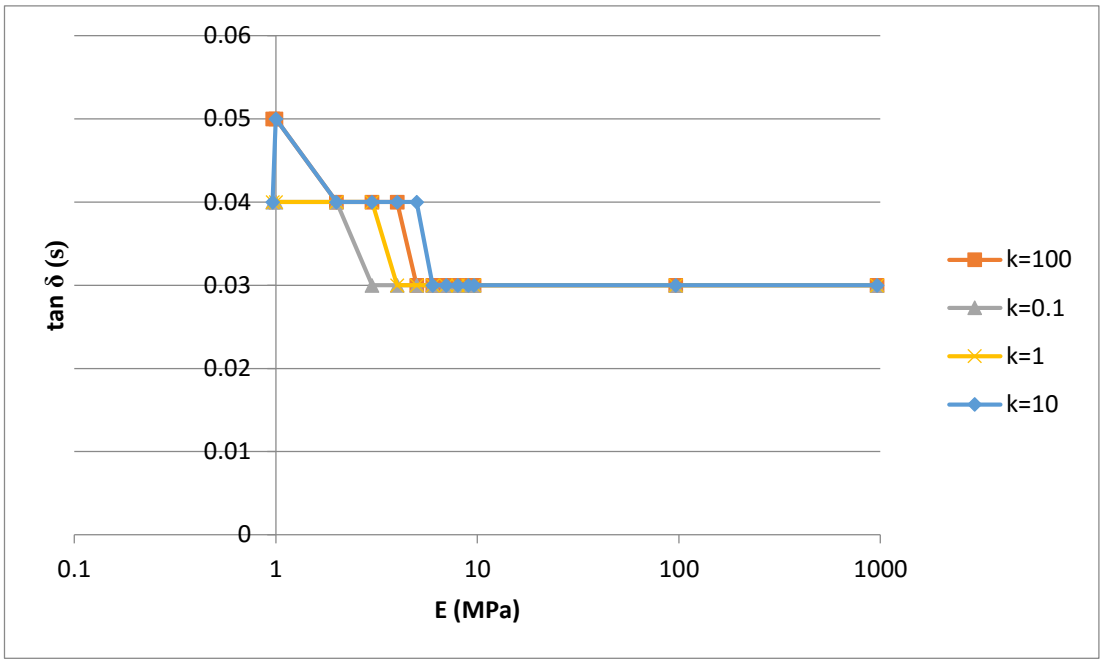


*Figure 39: The effect of hydraulic permeability on phase lag*

## Varying the Young's Modulus

The response of the system as the Young's Modulus is increased can be observed in Figure 40 and Figure 41 below. One may note that as the Young's Modulus increases the peak increases. As one can observe, the hydraulic permeability also affects the peak force; however, as the Young's Modulus is further increased, the peak force becomes more dependent on Young's Modulus and hydraulic permeability does not affect the response as much.

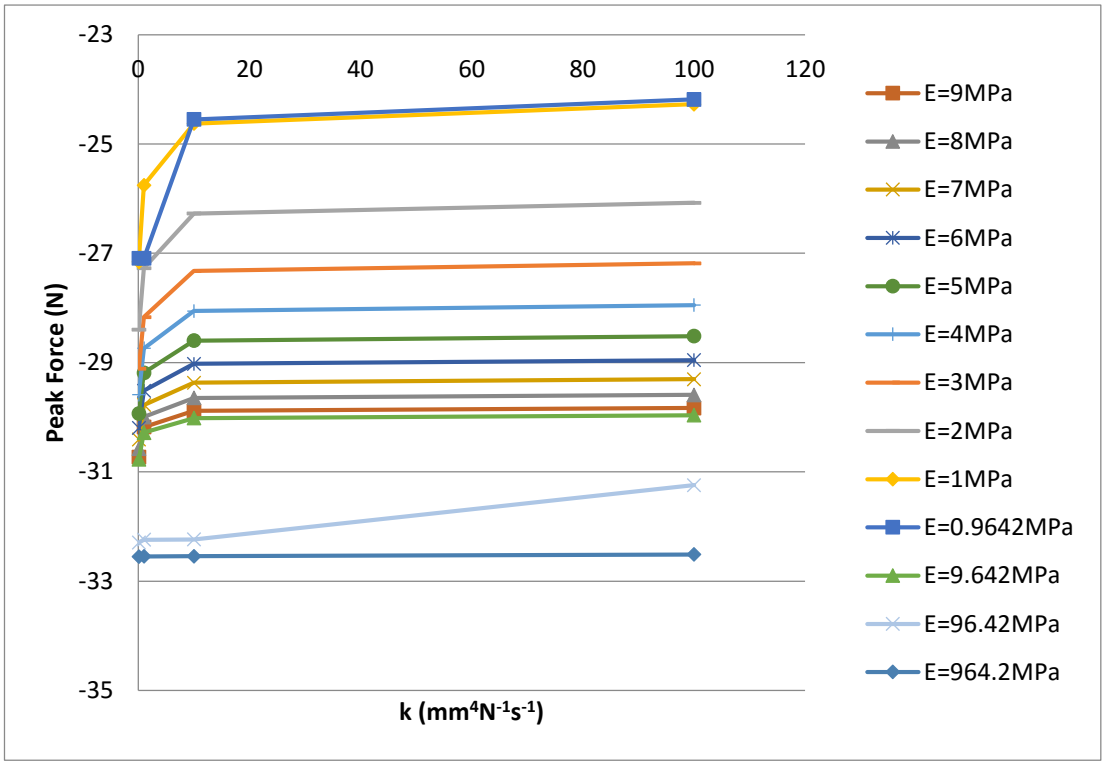


*Figure 40: The effect of Young's Modulus on peak force*

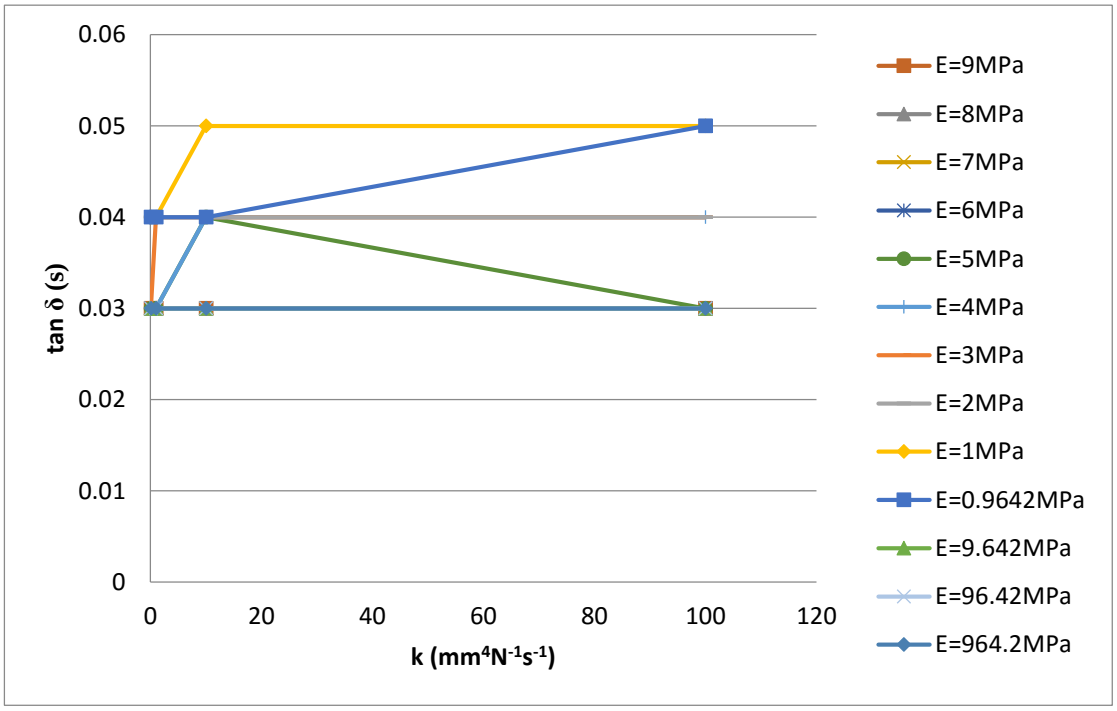


*Figure 41: The effect of Young's Modulus on phase lag*

### Varying the parameter $x/h$

The response of the system with variation of $x/h$ can be observed in Figure 43 below. One may note that as $x/h$ increases, the peak force applied by the platen on the material also increases. When the sphere is located relatively close to the centre of the cube ($0.4 < x/h < 0.6$), the peak force does not vary by much; however, as the sphere gets closer to the edges of the cube, the peak force changes by a larger amount. One may note that an increase in force magnitude can be observed when the sphere is shifted towards the bottom side ($x/h = 0.7$) whilst a decrease in force magnitude is observed

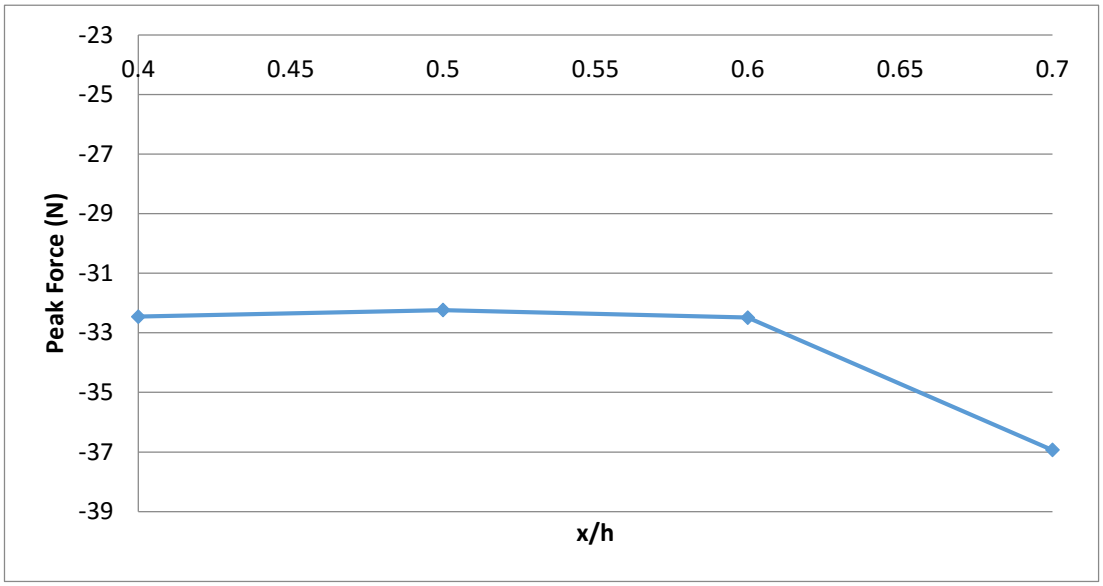


*Figure 43: The effect of x/h on peak force*

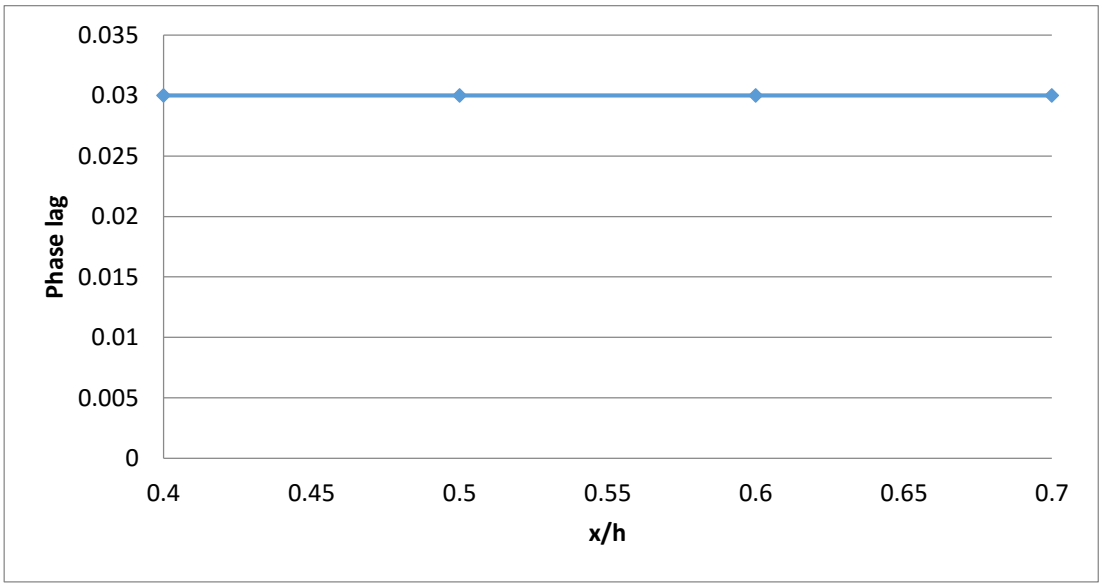


*Figure 42: The effect of x/h on phase lag*

when shifting the sphere towards the top side of the cube ($x/h = 0.3$). The phase lag ($\tan\delta$) can also be seen in Figure 42. This is observed to stay constant at a value of 0.03 seconds when $x/h \geq 0.4$ whilst a larger phase lag (0.05 seconds) can be observed for $x/h = 0.3$.

### Varying the Frequency of Oscillations

Figure 44 and Figure 45 describe the response of the materials across a range of frequencies. As can be observed, there is an increase in both peak force and phase lag as the frequency is increased. However, at a frequency of 8Hz, the peak force and phase lag both decrease and then start increasing again as the frequency increases further.

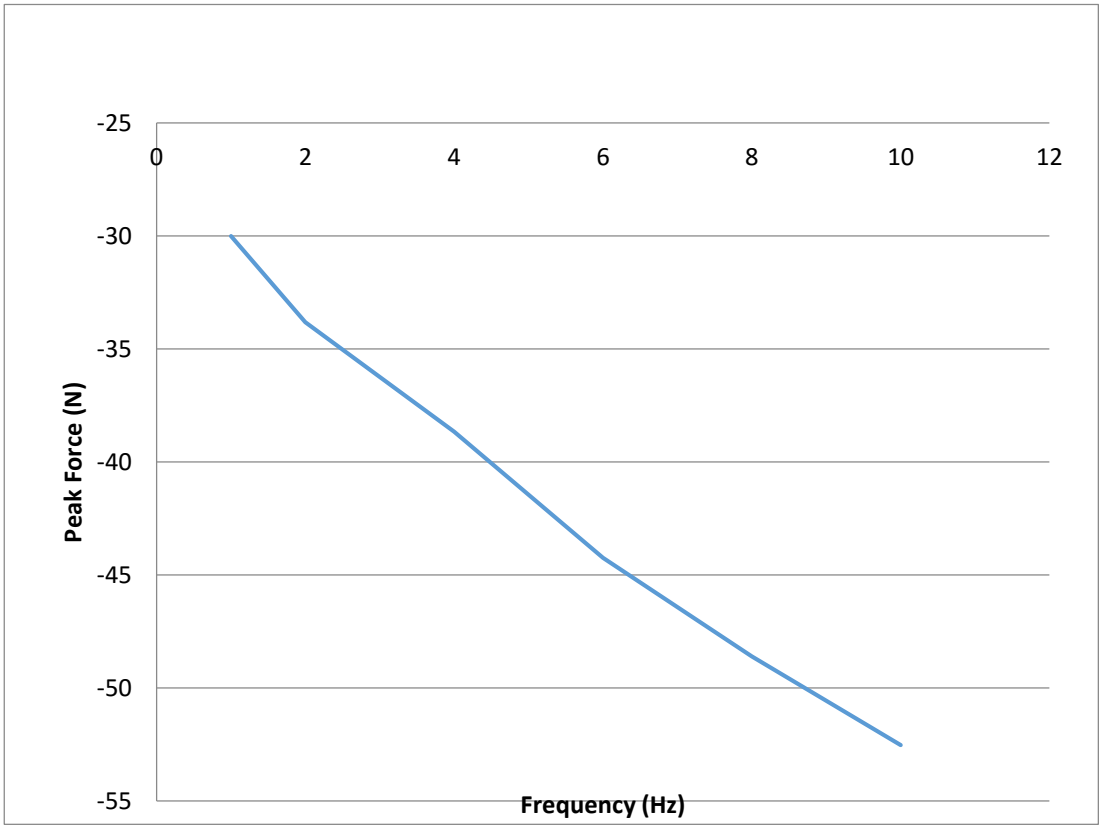


*Figure 44: The effect of frequency on peak force*

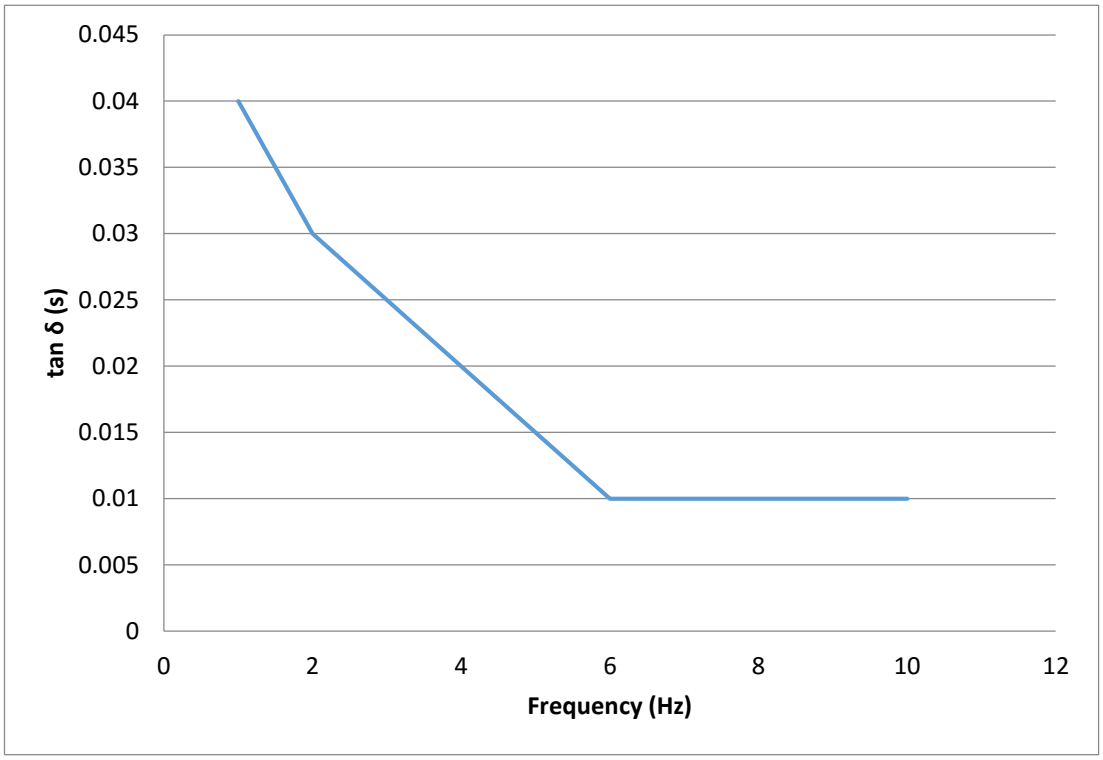


*Figure 45: The effect of frequency on phase lag*

# Discussion

As was proven in literature (Busby Graham A., 2013), the biphasic theory provides a good model for modelling such a situation. In this situation, there is essentially a section of material (sphere representing cancerous tissue) that has different properties than the rest of the material. This means that the response of the model is expected to change as the size and material properties of the sphere are varied.

## Ramp-Hold loading

### Varying the parameter $r/h$

As the parameter $r/h$ is varied within the model, the proportion of stiffer material (representing cancerous tissue) is increased. This means that in theory, the force applied by the platen should also increase as $r/h$ is increased. This response can be observed in Figure 25 and Figure 26.

Both the peak and equilibrium forces exhibit a similar response, the data can be compared to experimental data from literature (Busby Graham A., 2013). It was found that 95% of collagen hydrogels exhibit an equilibrium stress of $50 \pm 10Pa$ and a peak stress of $300 \pm 20Pa$. This means that a variation in peak force of $0.02N$ could be attributed to a difference in the gel rather than the presence of cancer. On the other hand, a variation in equilibrium force of $0.01N$ could be attributed to a difference in the gel as well. Since in these simulations, a force difference that was larger than $0.02N$ was detected between each case in both peak and equilibrium forces, it can be said that a change in volume ($r/h$) can indicate the presence of cancer within the hydrogel.

It is important to note that at very small radii of the sphere, there is barely any difference in response between the situation where there is no sphere and the situation where there is a very small sphere. This indicates that tumours of a smaller size would not be detected if such a method was used in order to diagnose cancers. Cancers that are at an early stage are usually small in size. This means that cancers having a low stage would not be identified using such a system. This provides a significant disadvantage since one of the most important aspects to cure cancer and recover from its effects is diagnosis at an early stage. Another disadvantage of such a system is that

it cannot diagnose cancers that have a high metastatic potential (i.e. have a high tendency to spread to other organs without creating lumps of large size). If this were the only system to be used for diagnosis, it could be particularly detrimental to a patient because it enables the cancer to develop further without any means of diagnosis.

### Varying the material properties ($k$ and $E$)

As hydraulic permeability of the sphere is increased, the resistance to fluid flow within the sphere is decreased, this means that a lower peak force magnitude is expected for higher hydraulic permeability. Equilibrium force magnitude is not expected to change when hydraulic permeability is changed; however it is expected that an equilibrium state is reached in a smaller time span since the lower restriction of fluid flow reduces the time where stresses induced by fluid pressure are acting. As can be seen in Figure 25Figure 30, the effect of a change in hydraulic permeability is most evident at a lower Young's Modulus and it can be observed that the results are similar to those expected.

As the Young's Modulus is increased, the material provides higher resistance to displacement. This means that both the peak force magnitude and equilibrium force magnitude required to deform the material should be higher when using a higher value of Young's Modulus. This can also be observed in Figure 25 and Figure 30 and further confirms that the situation was appropriately modelled.

The material properties of the sphere (modelling cancer) can be said to model different grades of tumour. This because as the grade of the tumour changes, its mechanical properties also change. The degree of difference of material properties for the sphere from those of the rest of the cube ($E = 0.9642MPa$ and $k = 100mm^4N^{-1}s^{-1}$) indicate a particular grad of tumour. From the results mentioned previously, it can be concluded that cancers of higher grade can be easily diagnosed using this method since it was found in literature (Suresh, 2007; Wenwei Xu, 2012) that cancers have higher stiffness than normal tissue.

It can be noticed that Young's Modulus has a higher impact on the response of the system rather than the hydraulic permeability. It is also important to note that in the literature, there was more emphasis placed on the stiffness of tumours (i.e. Young's Modulus) rather than on hydraulic permeability. This goes to show that the results

point in the right direction since they indicate that the response is also more dependent on the stiffness of the tumour.

This data can also be compared to experimental data from literature (Busby Graham A., 2013). It was found that 95% of collagen hydrogels exhibit an equilibrium stress of $50 \pm 10Pa$ and a peak stress of $300 \pm 20Pa$. This means that a variation in peak force of $0.02N$ could be attributed to a difference in the gel rather than the presence of cancer. On the other hand, a variation in equilibrium force of $0.01N$ could be attributed to a difference in the gel as well. In these simulations, a change in Young's Modulus of 1MPa, provides a change in response that affects the peak force by more than $0.02N$. This means that the stiffness of cancer can be used to identify cancers by analysing the peak force. On the other hand, the equilibrium response is barely affected and the difference in response is less than $0.01N$, this shows that the hydraulic permeability cannot be used to identify the presence of a cancer, since any difference in the response in this case might be attributed to a difference in properties of the gel rather than the presence of cancer.

### Time response for Varying Material properties

The time response in each case was very similar. The peak force was always reached at the same time, whilst an equilibrium state was also reached after a time period that was very similar in each case. The only exception to this observation were the cases with $k = 0.1mm^4N^{-1}s^{-1}$ and a Young's Modulus in the range $0.9642MPA \leq E \leq 2MPa$. In these cases, an equilibrium state took much longer to be reached as can be seen in Figure 32 in the results. This response could have been observed because the very low permeability makes the fluid phase flow more slowly within the biphasic material. On the other hand, the solid phase is more susceptible to deformation due to the low stiffness that it has. This means that as fluid is flowing through the pores, it will still strain the material despite the fact that it flows more slowly. This results in a longer dynamic state of deformation in the solid phase, thus the force exerted by the platen on the material takes longer to reach an equilibrium state during the hold phase.

It is important to note that for the case with $E = 0.9642MPa$ and $k = 0.1mm^4N^{-1}s^{-1}$, the number of steppe utilized by the solver was much larger than that used for other situations. Upon further investigation, in this case, it was found that the

solver encountered divergence issues, and steps of much shorter length had to be used in order to obtain convergence and eventually reach an equilibrium state during the hold phase. These issues could have occurred because it is very difficult to obtain a material with such a low hydraulic permeability whilst maintaining a very low Young's Modulus and retaining the same solid volume fraction (0.3%) and density ($1kgmm^{-3}$). This is because in a practical case, in order to obtain these values of hydraulic permeability and Young's Modulus, the density would have to be increased or otherwise the solid volume fraction should be increased in order to simulate a decrease in pore size (which would in turn help in making the low hydraulic permeability more realistic).

### Varying the parameter $x/h$

As can be observed in Figure 33 and Figure 35, the relationship of $x/h$ with peak force and equilibrium force indicates that for differences to be more identifiable, the biopsy should be placed towards the bottom section of the collagen hydrogel. Doing this will provide a difference in peak and equilibrium force that is higher than normal and thus simplify the detection of cancer.

Both the peak and equilibrium forces exhibit a similar response, the data can be compared to experimental data from literature (Busby Graham A., 2013). It was found that 95% of collagen hydrogels exhibit an equilibrium stress of $50 \pm 10Pa$ and a peak stress of $300 \pm 20Pa$. This means that a variation in peak force of $0.02N$ could be attributed to a difference in the gel rather than the presence of cancer. On the other hand, a variation in equilibrium force of $0.01N$coould be attributed to a difference in the gel as well. Since in these simulations, a force difference that was larger than $0.02N$ was detected between each case, it can be said that a change in the position of the cancer ($x/h$) can indicate the presence of cancer within the hydrogel; however, this is true only for the case when $x/h = 0.7$. In the other cases, the force required is either the same ($0.4 \leq x/h \leq 0.6$) or less than the normal response ($x/h = 0.3$)

## Sinusoidal loading

### Varying the parameter $r/h$

As the parameter $r/h$ is varied within the model, the proportion of stiffer material (representing cancerous tissue) is increased. This means that in theory, the peak force

applied by the platen should also increase as $r/h$ is increased. This response can be observed in Figure 36.

Both the peak and equilibrium force and phase lag exhibit a similar response shown Figure 36 and Figure 37, the data can be compared to experimental data from literature (Busby Graham A., 2013). It was found that 95% of collagen hydrogels exhibit an equilibrium stress of $50 \pm 10Pa$ and a peak stress of $300 \pm 10Pa$. This means that a variation in force that varies by $0.02N$ could be attributed to a differences in the gel rather than the presence of cancer. Since in these simulations, a force difference that was larger than $0.02N$ was detected in most cases, it can be said that a change in volume ($r/h$) can indicate the presence of cancer within the hydrogel.

It is important to note that at very small radii of the sphere($r/h \leq 0.05$), there is barely any difference in peak force magnitude response between the situation where there is no sphere and the situation where there is a very small sphere (this difference is less than $0.02N$). This indicates that tumours of a smaller size would not be detected if such a method was used in order to diagnose cancers since such a difference could be attributed to a difference in gel. In this case however, the phase lag can be used to determine whether a cancer is present. This because a large difference in phase lag can be observed at small radii. This means that cancers that are at an early stage can be diagnosed by using a sinusoidal loading instead of ramp-hold loading. This provides a significant advantage over ramp hold loading experiments because with a small modification to the time-response of the displacement of the platen, cancers may be diagnosed at an earlier stage. A disadvantage of such a system is the need for highly sensitive apparatus in order to measure the phase lag. This would require a recording system with high frequency so that small phase lags can be measured.

### Varying the material properties ($k$ and $E$)

As the hydraulic permeability of the sphere is increased, the resistance to fluid flow within the sphere is decreased, which means that a lower peak force magnitude is expected for higher hydraulic permeability. As can be seen in Figure 38 and Figure 41, the effect of a change in hydraulic permeability is most evident at a lower Young's Modulus and it can be observed that the results are similar to those expected.

As the Young's Modulus is increased, the material provides higher resistance to displacement. This means that both the peak force magnitude and equilibrium force magnitude required to deform the material should be higher when using a higher value of Young's Modulus. This can also be observed in Figure 38**Figure** 41and further confirms that the situation was appropriately modelled.

In both cases, a phase lag can be attributed to a difference in material properties; no conclusion can be made about this since no obvious trend could be observed.

The material properties of the sphere (modelling cancer) can be said to model different grades of the tumour. This because as the grade of the tumour changes, its mechanical properties also change. The degree of difference of material properties for the sphere from those of the rest of the cube ($E = 0.9642MPa$ and $k = 100mm^4N^{-1}s^{-1}$) indicate a particular grade of tumour. From the results mentioned previously, it can be concluded that cancers of a higher grade can be easily diagnosed using this method since it was found in literature (Suresh, 2007; Wenwei Xu, 2012) that cancers have higher stiffness than normal tissue. It can be observed that Young's Modulus has a higher impact on the response of the system rather than the hydraulic permeability. It is also important to note that in literature, there was more emphasis placed on the stiffness of the tumours (i.e. Young's Modulus) rather than on hydraulic permeability. This goes to show that the results point in the right direction since they indicate that the response is also more dependent on the stiffness of the tumour.

When this data was compared to experimental data from literature (Busby Graham A., 2013), it was found that 95% of collagen hydrogels exhibit an equilibrium stress of $50 \pm 10Pa$ and a peak stress of $300 \pm 10Pa$. This means that a variation in force that varies by $0.02N$ could be attributed to a differences in the gel rather than the presence of cancer. It can be said that at low values of Young's Modulus ($E < 96.42MPa$), the hydraulic permeability can be used to detect cancer, however this cannot be said for higher values of Young's Modulus. In such a case, the Young's Modulus should be used to identify the presence of cancer.

### Varying the parameter $x/h$

As can be observed in Figure 42 and Figure 43, placing the biopsy at the lower part of the hydrogel gives a better peak force difference. This can be used to identify the presence of cancer since it is larger than $0.02N$ and such a difference in force cannot be attributed to a variation in gel properties (Busby Graham A., 2013). In such a case, one would also observe a decrease in phase shift, however, this is still detectable and should not pose any problems in the identification of cancer.

### Varying the Frequency of Oscillations

As can be observed in Figure 44, the peak force magnitude increases as frequency increases. This means that using a higher frequency of oscillations would help in better detecting cancerous cells within the material. Figure 45 also whows the phase lag. As one may observe, this is observed to decrease as frequency increases, and it reaches a constant value of $0.01s$ at a frequency of $6Hz$. This means that phase lag would be more difficult to detect as frequency increases; however, since there was no obvious relationship between the material properties of the sphere and the phase lag, it would be of higher significance if the Young's Modulus were to be used to diagnose cancer in conjunction with a high frequency of oscillation.

## Conclusions

In this study, the mechanical properties of cancer were researched, and the current ways of diagnosing cancer were identified. The study was focused on using the mechanical characteristics of cancer cells as a method of diagnosis for the presence of these cells, and this method was tested by using finite element simulations. FEBio was used to model cancerous cells cultured in a collagen hydrogel and two different loading profiles (ram-hold and sinusoidal) were used to examine the response. Different parameters were varied in order to observe their effect on the response of the system and these effects were studied to demonstrate any significance.

From this study it can be concluded that both methods of loading have various advantages: ramp-hold loading is simpler in nature, and can provide more accurate results for increased sizes of biopsies. On the other hand, sinusoidal loading can provide accurate results even for small biopsies by using both the peak force readings and the phase lag. It can be also carried out in less time than the ramp-hold (2 seconds are enough for sinusoidal whereas 10-14 seconds are required for ramp-hold loading).

A larger size of the biopsy was found to be more useful in detecting cancer with the best response obtained at value of $r/h = 0.3$. This signifies that the biopsy should be as large as possible. A difference in Young's Modulus that is larger than $1MPa$ can provide a significant difference in force response in all cases, whilst varying the hydraulic permeability does not give a significant difference in force response for values of Young's Modulus that are larger than $96.42MPa$.

It is important to note that although this method has the potential to detect cancer, it is still not as accurate as other methods of testing which can provide the practitioner with the type of cancer, grade, and stage. This method can only provide confirmation whether or not there is a tumour within a biopsy. The stage of the cancer can be detected from the response; however, no information can be obtained about the grade of the cancer or about the tissue that it originated from. This method also does not provide any information as to methods for treatment in contrast to other methods that are currently used.

After further study, analysis and verification, such a method would be recommended as a preliminary test before carrying out other tests that are more time consuming.

Such a test would be ideal due to the fact that it does not take long and can be carried out whilst the patient has to wait only a short while to obtain the test results. If tests from this method are positive, further testing should be carried out using the standard testing techniques in order to obtain more in depth information about the cancer.

# References

Canadian Cancer Society - Anatomy and Physiology of Soft Tissue.

Krames Online - Percutaneous Diagnosis of Lung, Chest Problems.

National Cancer Institute at the National Institute of Health - Cancer Staging Fact Sheet.

(Online) - Animal cell.

Openstax College - Anatomy and Physiology - 2013.

Worldwide Cancer Research - Cancer Basics.

Allan), R.A.W.R., 2007. The Biology of Cancer. Garland Sciences, New York.

Baker, E.L., Lu, J., Yu, D., Bonnecaze, R.T., Zaman, M.H., 2010. Cancer Cell Stiffness: Integrated Roles of Three-Dimensional Matrix Stiffness and Transforming Potential. Biophysical Journal 99, 2048-2057.

Barry, C.A.A.a.S.A., 2010. Cancer: Basic Science and Clinical Aspects. John Wiley & Sons Ltd., Oxford.

Busby Graham A., M.H.G., Simon P. MacKay, Philip E. Riches, 2013. Confined compression of collagen hydrogels. Journal of Biomechanics, 837-840.

Butcher, D.T., Alliston, T., Weaver, V.M., 2009. A tense situation: forcing tumour progression. Nat Rev Cancer 9, 108-122.

Casares, L., Vincent, R., Zalvidea, D., Campillo, N., Navajas, D., Arroyo, M., Trepat, X., 2015. Hydraulic fracture during epithelial stretching. Nat Mater 14, 343-351.

Christian Frantz, K.M.S.a.V.M.W., 2010. The extracellular matrix at a glance. Journal of Cell Science, 4195-4200.

Cox, T.R., Erler, J.T., 2011. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Disease Models & Mechanisms 4, 165-178.

Faguet, G.B., 2008. The War on Cancer - An Anatomy of Failure - A Blueprint for the Future. Springer, pp. 5-58.

Farrell, M.D.a.P.E.R., Year Poisson's Ratio of Nucleus Pulposus Tissue: Comparison of Experimental Results with a Biphasic Poroviscoelastic Finite Element Model. In Euromech 534 Colloquium: Advanced Experimental Approaches and Inverse Problems in Tissue Biomechanics, 2012-05-29 - 2012-05-31, St Etienne.

Fenner, J., Stacer, A.C., Winterroth, F., Johnson, T.D., Luker, K.E., Luker, G.D., 2014. Macroscopic Stiffness of Breast Tumors Predicts Metastasis. Sci. Rep. 4.

Forgacs, G., Foty, R.A., Shafrir, Y., Steinberg, M.S., 1998. Viscoelastic properties of living embryonic tissues: a quantitative study. Biophysical Journal 74, 2227-2234.

Kandice R. Levental, H.Y., Laura Kass, Johnathon N. Lakins, Mikala Egeblad, Janine T. Erler, Sheri F.T. Fong, Katalin Csiszar, Amato Giaccia, Wolfgang Weninger, Mitsuo Yamauchi, David L. Gasser, and Valerie M. Weaver, 2009. Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin signaling. Cell 5, 891–906.

Karol Miller, D., 1998. Modelling Soft Tissue Using Biphasic Theory — A Word of Caution. Computer Methods in Biomechanics and Biomedical Engineering 1, 261-263.

Ko, A.H., 2008. Everyone's Guide to Cancer Therapy, 5 ed.

Lu, P., Weaver, V.M., Werb, Z., 2012. The extracellular matrix: A dynamic niche in cancer progression. The Journal of Cell Biology 196, 395-406.

Maas S.A., E.B.J., Ateshian G.A., Weiss J.A., 2012 FEBio: Finite elements for biomechanics. Journal of Biomechanics 1, 011005.

Mayeux, R., 2004. Biomarkers: Potential Uses and Limitations. NeuroRx 1, 182-188.

Moeendarbary, E., Valon, L., Fritzsche, M., Harris, A.R., Moulding, D.A., Thrasher, A.J., Stride, E., Mahadevan, L., Charras, G.T., 2013. The cytoplasm of living cells behaves as a poroelastic material. Nat Mater 12, 253-261.

Morgan, E.F., Longaker, M.T., Carter, D.R., 2006. Relationships between tissue dilatation and differentiation in distraction osteogenesis. Matrix biology : journal of the International Society for Matrix Biology 25, 94-103.

Mow, V.C., Kuei, S. C., Lai, W. M. and Armstrong, C. G. , 1980. Biphasic Creep and Stress Relaxation of Articular Cartilage in Compression: Theory and Experiments. Journal of Biomechanics 1, 73-84.

Orell, S.R., 2012. Orell & Sterrett's Fine Needle Aspiration Cytology, 5 ed. Elsevier.

Prendergast P. J., v.D.W.D.a.K.J.-H., 1996. 'A Comparison of Finite Element Codes for the Solution of Biphasic Poroelastic Problems', Proceedings of the Institution of Mechanical Engineers. Journal of Engineering in Medicine, Part H 2, 131-136.

Ragsdale, G.K., Phelps, J., Luby-Phelps, K., 1997. Viscoelastic response of fibroblasts to tension transmitted through adherens junctions. Biophysical Journal 73, 2798-2808.

Simon, B., 1991. Poroelastic Finite Element Models in Biomechanics — an Overview, in: Williams, K.R., Toni, A., Middleton, J., Pallotti, G. (Eds.), Interfaces in Medicine and Mechanics—2. Springer Netherlands, pp. 279-288.

Society, A.C., Testing Biopsy and Cytology Specimens for Cancer.

Souhami, R.L., Tobias, J.S., 2007. Cancer and its Management. Wiley-Blackwell.

Suresh, S., 2007. Biomechanics and biophysics of cancer cells. Acta Biomaterialia 3, Pages 413–438.

Tavel, K.S.a.J.A., 2011 What are Biomarkers. Current opinion in HIV and AIDS 5, 463–466.

UK, C.R., Cancer Statistics for the UK.

Wenwei Xu, R.M., Byungkyu Kim, Lijuan Wang, John McDonald, and Todd Sulchek, 2012. Cell Stiffness Is a Biomarker of the Metastatic Potential of Ovarian Cancer Cells. Plos One.