BONE GROWTH AND REMODELING: FROM CONCEPT TO SIMULATIONS

by

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Abstract

Bone growth and remodeling are complex phenomena that are influenced by a variety of factors including mechanical stimuli. However, it is still unclear how to identify and quantitatively characterize the mechanical stimuli responsible for bone cell growth. The objective of this study is to design and simulate an experimental apparatus to cyclically apply pressure and shear stresses to bone cells and observe their growth (or lack thereof) as a function of the applied loads.

Acknowledgements

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Chapter 1

Introduction

I Background

There is a growing interest in determining the effect of mechanical stimuli on bone growth and remodeling as these phenomena may have a strong effect on the life of a prosthetic implant[6]. During day to day life our bones experience various loading conditions[2]. Often, the effect of mechanical stimuli on the bone surrounding an implant are modeled using continuum mechanics and finite element modeling [4, 9, 12]. For example, Schmitz et. al [5] investigate and compare the effect of various mechanical factors on bone growth. To do this, their work compares various mechanical signals involving strains and strain energy density and apply these stimuli in finite element simulations analyzing the hip implant in a single patient.

The work from Schmitz et. al [5] also shows that while mechanical stimuli do play a key role in bone growth, bone development and remodeling depends also on other factors that are not mechanical. Some of these factors include age, gender, and the level of physical exercise that a patient participates in. Consequently, corrective ratios for the mechanical stimuli should be utilized to best match rates of re-absorption and deposition of bone material for a representative subject in each patient age group.

The article states that the currently accepted mechanical signals can be broken into three

distinct groups.

1. Strain indicators (max shear strain, minimal principal strain, and equivalent strain);

2. Strain energy indicators (per unit mass and per unit volume);

3. Compression based dilatational strain indicators.

These indicators are then applied in a finite element analysis to predict the bone growth and loss in various regions of the post-implant femur. It is worthy of note that none of the remodeling signals included within this paper specifically account for the effects of interstitial fluid shearing despite its seemingly significant role and possibly unique effects on bone remodeling as highlighted in [14].

From the patient data available, Schmitz et. al. conclude that deviatoric and strain energy indicators are the best candidates to account for the mechanical stimuli, while dilational strain signals appeared to have the least correlation to the actual remodeling. However, the authors point out that the data is too limited to arrive at any definite conclusions about the accuracy and/or significance of these results.

In their experimental work, Abubakar et. al[3] monitor bone growth as a function of cyclical and non-cyclical hydrostatic pressures using fetal chick femurs. The conclusions drawn from this paper support other works by stating that cyclical loading is required for effective bone growth. Additionally, the paper shows that the frequency of loading does play a role. Based on these findings, in designing our apparatus, we ensure that cyclic loading can be applied. The magnitude of stress applied along with the frequency of application appears to be a significant factor in bone development and should be able to be controlled within the apparatus being developed with an emphasis on the magnitudes rather than frequencies of application.

Perfusion bioreactors are also promising apparatus to study the effects of shear loading on bone absorbtion and deposition. “The Role of Perfusion Bioreactors in Bone Tissue Engineering” by Gaspar, Diana Alves, et al.[11] discusses the various methods of stimulating

bone structure growth and bone cell deposition. Three separate categories of bioreactors are discussed: Spinner Flasks, Rotating Wall Vessels, and Perfusion Bioreactors. Spinner flasks are described as a simple setup with growth medium and bone cells within a flask, which provides mechanical stimuli through the mixing of the liquid. Experiments involving spinner flasks generally show defined cell growth but the nutrients are not always replenished and a buildup of byproducts sometimes occurs. Additionally spinner flasks, by design, cause cell growth generally at the edge of the flask where the shear stress are the highest. Rotating wall vessels offer more control over oxygen and nutrient levels than spinner flask setups as they typically produce less turbulence and maximize the shear stress. This review suggests that, as the study of bone tissue engineering continues, research has began to utilize perfusion bioreactors to better gain control over the various mechanical conditions such as fluid shearing, hydrostatic pressure, and loading cycle frequency with the goal of maximizing bone growth.

Perfusion bioreactors are presented in [11] as the best choice in many studies as they appear to provide the most uniform cell growth of the three previously mentioned methods while retaining relative mechanical simplicity. At it's most basic level, a perfusion bioreactor contains a pump which circulates and replenishes nutrients in a growth medium. Fluid travels through a porous structure or scaffolding in what is called a "profusion chamber". Sometimes a constant flow rate is provided by the pump, but in other investigations the pump provides pulsatile flow or a fully reversing oscillatory flow. It must be noted though that the geometry of the profusion chamber at this point and time is by no means standardized and there is still much discussion on the optimal geometry for bone growth.

It is emphasized within [11] that despite the simple construction of a bioreactor, many factors affecting bone growth must be taken into consideration including: cycle frequency (or lack thereof), shear stress, flow rate, pore size, and chamber geometry. The article provides summarized data on the design of each apparatus and the typical forces that are experienced in each design. Pore size of the substrate in these studies varies greatly from 17μm to

1000μm, flow rates in bioreactors varies from from 0.01ml/min to 10ml/min, shear stress (when reported) was approximated to be anywhere from 0.02dyn/cm^2 to 4.3dyn/cm^2 and flow frequency between 0 and 20 Hz. It is concluded in [11] that, in part due to the amount of factors at play, much work is still needed to understand the optimal growth conditions and setup for profusion bioreactors when studying bone tissue growth. The designs created through our research will potentially be able to answer some of the questions regarding how mechanical factors such as hydrostatic pressure and shear stress affect bone growth and development in isolation.

II Motivation

An accurate understanding of the mechanical stimuli required for bone growth is of notable importance when determining the effect of a prosthetic implant on the bone tissue at and around the implant. As the understanding of the specific mechanisms grows, modern implant designs have been created and monitored with the assistance of finite element analysis. Such evaluations allow researchers, physicians, and physical therapists alike to predict post-operation bone density changes more effectively and to identify problems along with potential solutions. Improvement to the understanding of bone growth allows for innovative implant conceptualization, simulation, and design iteration without the need for costly, time-consuming, and sometimes unpredictable experimental clinical procedures [13].

Detailed understanding of the mechanical signals which stimulate bone growth and remodeling is an essential part of the finite element analysis process when attempting to determine how a patients body will adapt to the foreign implant. Through finite element analysis, the relevant mechanical states involving localized strains and stresses can be determined within a mechanical implant and the surrounding bone structure. While many studies have set out to characterize the growth behavior of bone tissue through trials and experiments, both ex-vivo and en-vivo, a large portion of the trials have focused on uniform compression and tension (e.g., [1, 15]) or observed patient-specific situations [5, 7]. Unfortu-

nately, there is still considerable uncertainty when considering the role that specific localized stresses, such as shear and hydrostatic pressure, have in bone development.

In the interest of only focusing on the shear and hydrostatic stresses, the designed apparatus utilizes fluid flow to generate shear stresses along with a hydrostatic pressure. Two setups are to be created such that the shearing effects are maximized in one and minimized in the other, while maintaining consistent hydrostatic loading across configurations. This design will allow to differentiate the role of shear stresses in the growth of bone cells in the presence of similar hydrostatic loading.

The isolation of specific stresses in a controlled and repeatable process will allow for more accurate understanding and subsequent modeling of the mechanical stimulus. The results gained from the bone density measurements [10] and their subsequent analysis have the potential to improve the current computational models by including more accurate mechanical growth factors [14]. Improving the simulation of growth signaling can improve the quality of life of those requiring bone implants by paving the way to improve current implant technology and prediction of an implant life in a patient.

Chapter 2

Apparatus Design

I Design Theory

The experimental apparatus developed in this work utilizes fluid flow at an interface to create the desired shear stress. A standard bone growth media [3] is to be cycled through a cylinder that contains a coated substrate containing bone cells such as that seen in [8]. The sample is placed at the bottom of the cylinder where the cell replicate or die as a function of the applied mechanical stimulus (Figure 2.1). The proposed apparatus will consist of a flow system, which will produce similar loading conditions to those found within both perfusion bioreactors [11] and other hydrostatic loading devices [1].

In order to produce the desired shear stress, the apparatus was designed to produce a jet of fluid that contacts bone cells on the substrate. The fluid then travels in line with the sample and creates the desired mechanical stimulus on the bone cells.

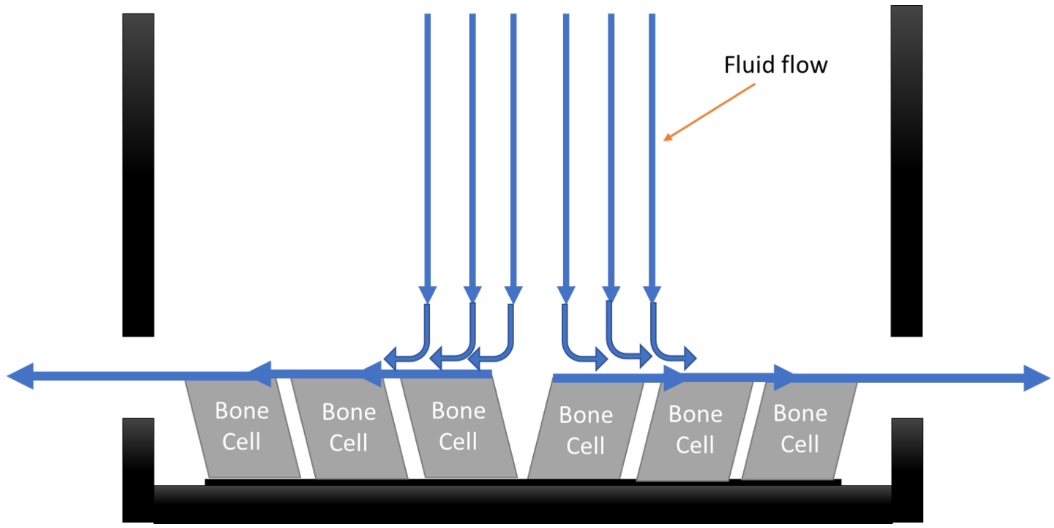


Figure 2.1: Design concept

With interest in determining what effect fluid shearing and hydrostatic loading have, multiple configurations are created which will attempt to minimize fluid shearing in one while maximizing it in the other. The objective of the apparatus design is to maintain all other mechanical and environmental factors as consistent as possible across experiments.

The first configuration of the design (Figure 2.2 - left) consists of a recirculating pump that sends growth media in and out of a cylinder through a piston. The low placement of the openings generate the desired shear stress due to the viscus effects of the fluid [11] as it moves across the surface of the bone microstructure.

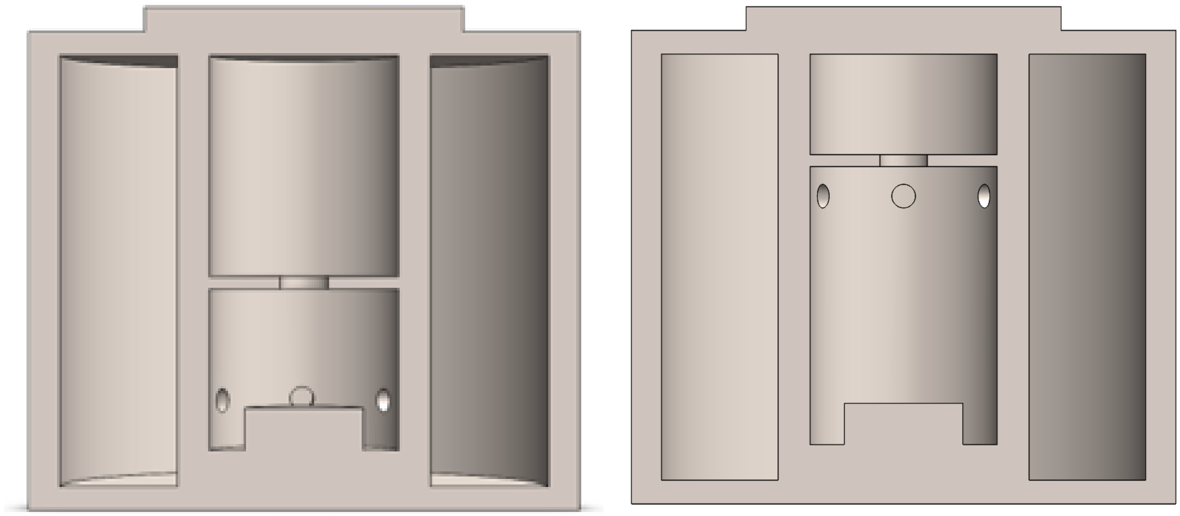


Figure 2.2: Configuration with hydrostatic loading and fluid shearing (left) and mainly hydrostatic loading (right).

The Second mechanism (Figure 2.2 - right) aims to study an alternating hydrostatic pressure scenario with minimal shear stresses exerted on the bone cells. This apparatus consists of a recirculating pump that sends growth media through a set of spaced holes in such a way that the shear stress generated by the fluid flow is minimized while the internal hydrostatic pressure and fluid mixing remains similar to the first configuration.

II Fluid Motion Design

The desired fluid flow paths are depicted in Figures 2.3 and 2.4, showing how the mechanical environment will be created. For both configurations, the goal is to have control over the shear stress applied and all other factors to remain as constant as possible.

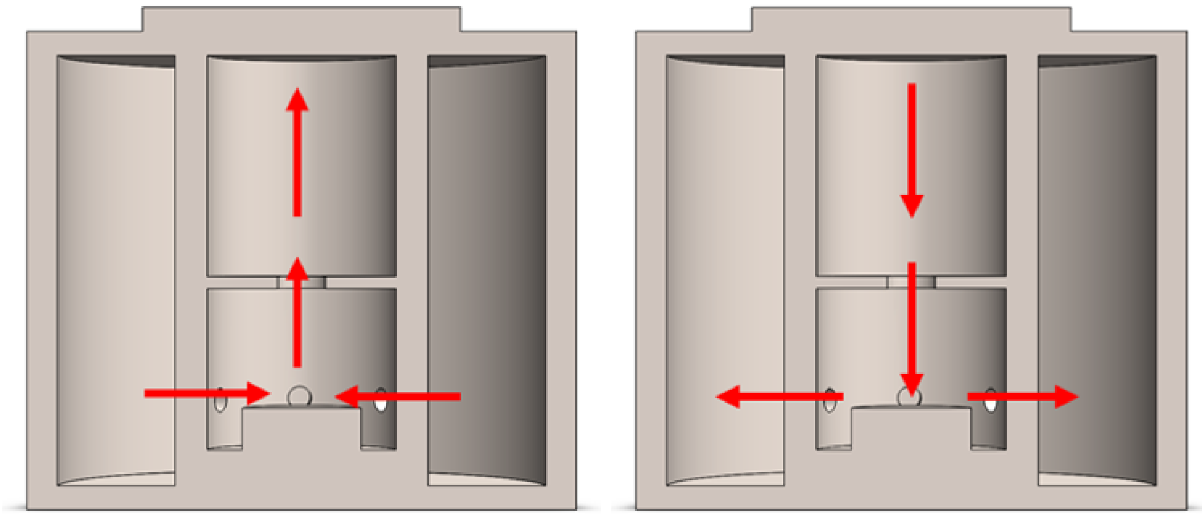


Figure 2.3: Desired flow path to maximize fluid shearing at bone interface: upward piston motion (left) and downward piston motion (right).

The first configuration (Figure 2.3) has holes in the lower portion of the chamber, with the ideal flow being parallel to that of the bone cell sample, hence causing substantial fluid shearing to take place near the sample [15]. Configuration 2 (Figure 2.4), due to it's higher

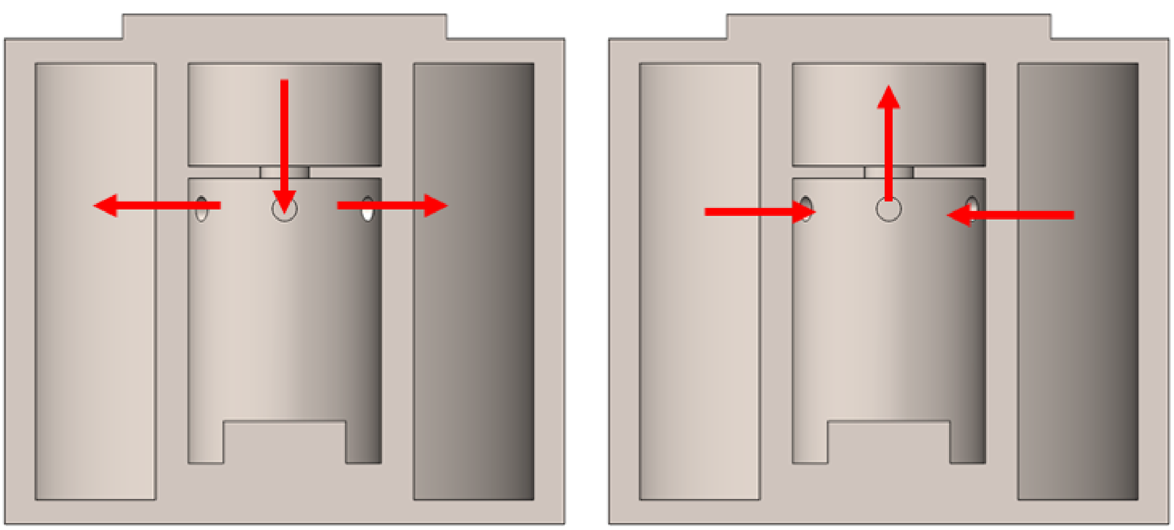


Figure 2.4: Desired flow path to minimize fluid shearing at bone interface: downward piston motion (left) and upward piston motion (right).

hole placement, has an expected fluid flow path that will provide minimal fluid shearing to the bone cells, while providing an adequate amount of grow medium mixing and similar hydrostatic pressure to configuration 1.

Chapter 3

Simulations

I Overview

A refined and a simplified surface mesh were created in SOLIDWORKS consisting of the same core dimensions of the full assembly. The surface mesh was then exported from SOLIDWORKS into StarCCM+ via a model in the IGS format. Within StarCCM+, analysis parameters were assigned as shown in Figure 3.1.

Figure 3.1: Simulation parameters

A basic mesh refinement study was then conducted and once completed, it was established that the current mesh was appropriate for the needs of the study. Each simulation was then

ran for 1000 iterations to ensure it reaches a steady state. Finally, two simulations were completed per configuration, one for the piston moving upwards and one for the piston moving downwards, resulting in a total of four simulations.

II Boundary Conditions

The assigned boundary conditions remain identical across all simulations with a constant velocity of 5mm/s for the piston downward motion and −5mm/s for the piston upward motion. Both simulations place the piston location at a height corresponding to approximately the middle of the piston's stroke while a relative pressure of 0Pa was assigned as boundary condition at the top most part of the fluid container (Figure 3.2). All other surfaces are considered rigid and stationary.

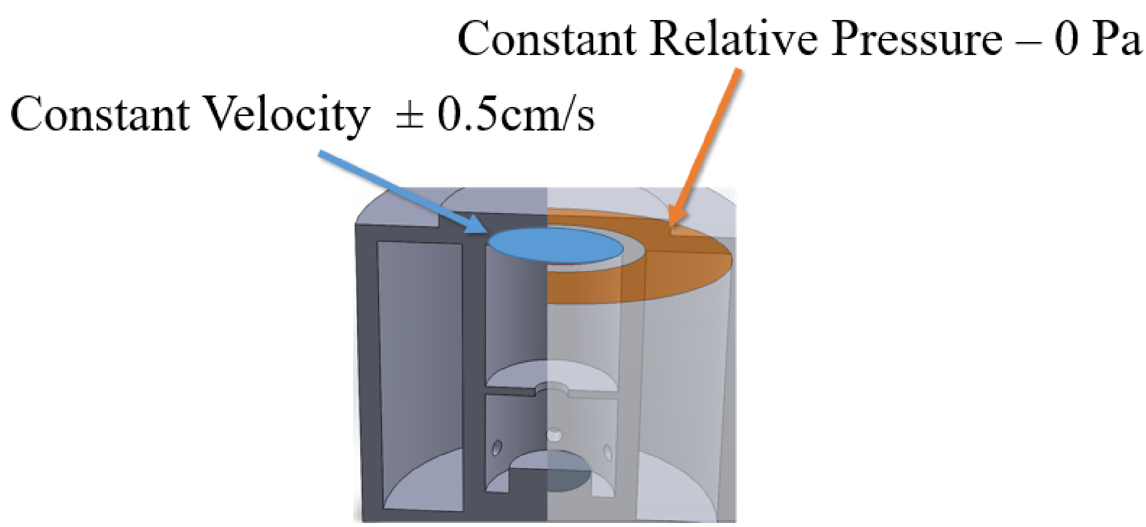


Figure 3.2: Boundary conditions

III Material Properties

IAPWS-IF97 (water) was selected for the fluid medium, as at this point in time the growth media's properties are still undetermined. In the future, depending on the properties, especially the viscosity, simulations can be modified with relative ease in order to better represent

the actual experiments.

IV Meshing

Meshing was completed using the following models:

1. Prism Layer Mesher to ensure accurate and high resolution close to boundary surfaces and bone cell surface.

2. Surface Wrapper to ensure an accurate surface mesh.

3. Surface Remesher to then simplify the surface mesh.

4. Extruder to generate the desired 3D mesh.

5. Trimmer to clean mesh and improve mesh quality.

All meshing settings are left to their default values except for the following parameters:

1. Maximum cell size was decreased from 1000 percent to 200 percent of base size;

2. Base cell size was modified to be 0.25mm.

Cross sections of the meshes are shown in Figure 3.3. Note the smaller mesh size close to the walls. This is crucial, especially near the substrate surface since the local shear and hydrostatic stresses are the primary quantities of interest.

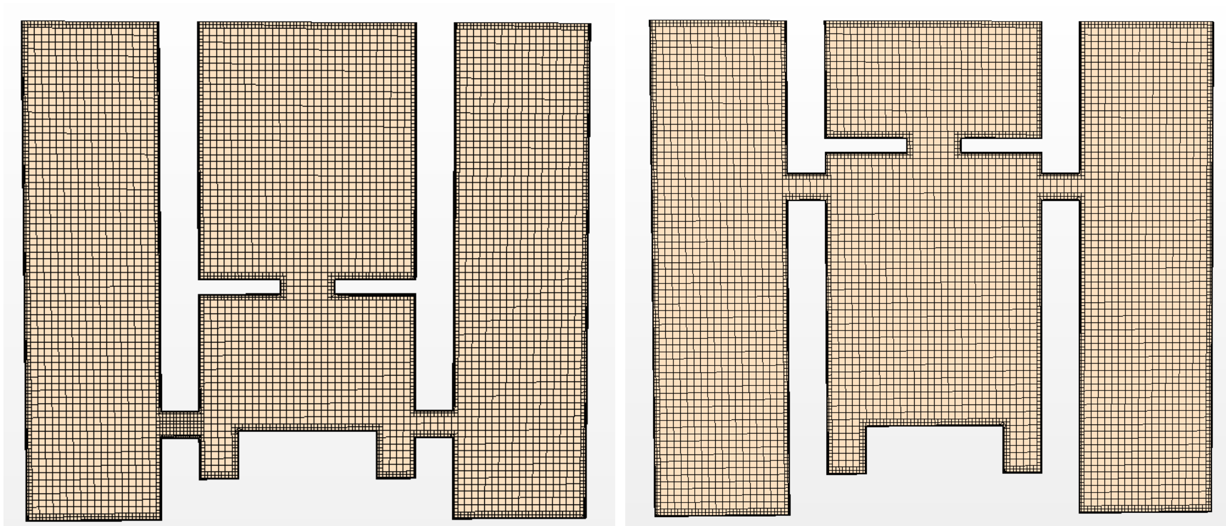


Figure 3.3: Fluid mesh - high shear configuration (left) and low shear configuration (right)

As expected, both meshes, aside from the locations of the holes, look very similar in both size and structure.

IV.1 Mesh Convergence Study

A mesh convergence study was conducted to ensure that the mesh size and configuration were sufficient for this study. To do this we decreased the mesh size from 0.25mm to 0.15mm, with the largest element being only two times the base mesh size.

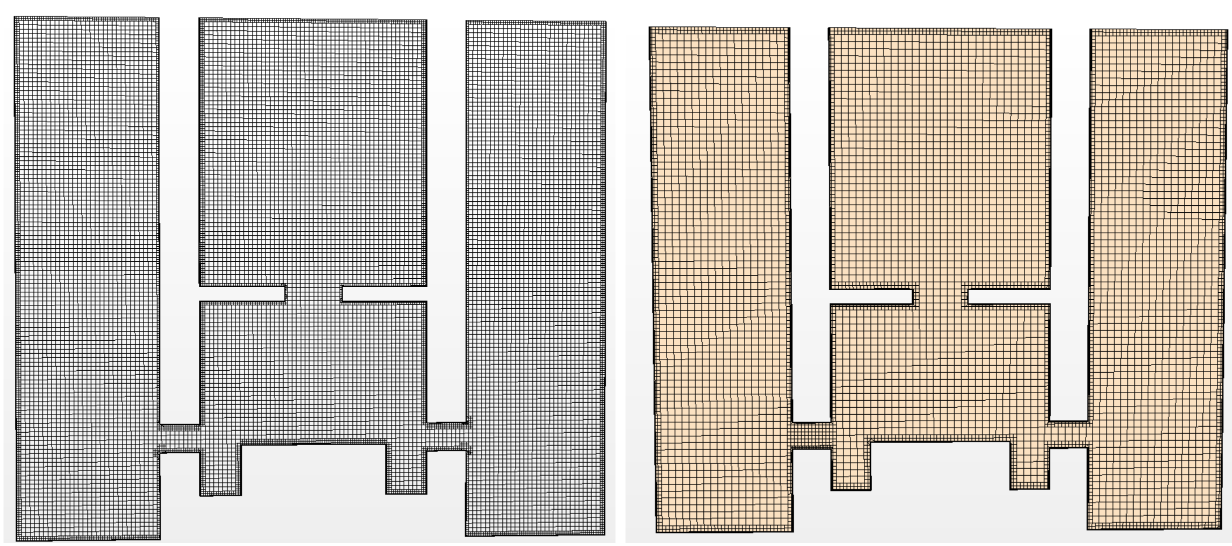


Figure 3.4: Fluid mesh - Refined (left) and original (right)

Visual and numerical comparisons were carried out among the values computed with the original and refined meshes. As visible in Figure 3.5, the relative pressure values within the different areas of the apparatus are similar in the original and refined simulations. Similarly, the shear stresses at the bone interface in the original and refined meshes are similar (Figure 3.6). While the sample is discretized using a finer resolution in the refined mesh, the refined mesh does not appear to influence significantly the quantities of interest in these simulations and therefore the original mesh is deemed sufficient at this point.

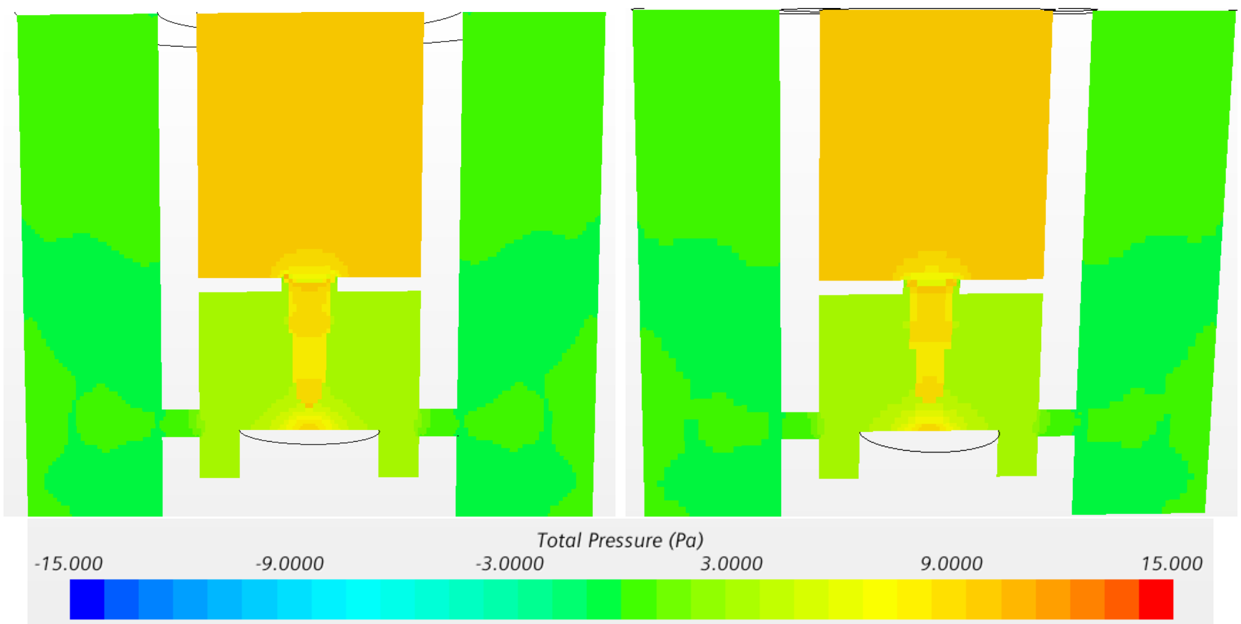


Figure 3.5: Apparatus relative pressure comparison. Refined mesh (left) and original mesh (right) for high shear configuration.

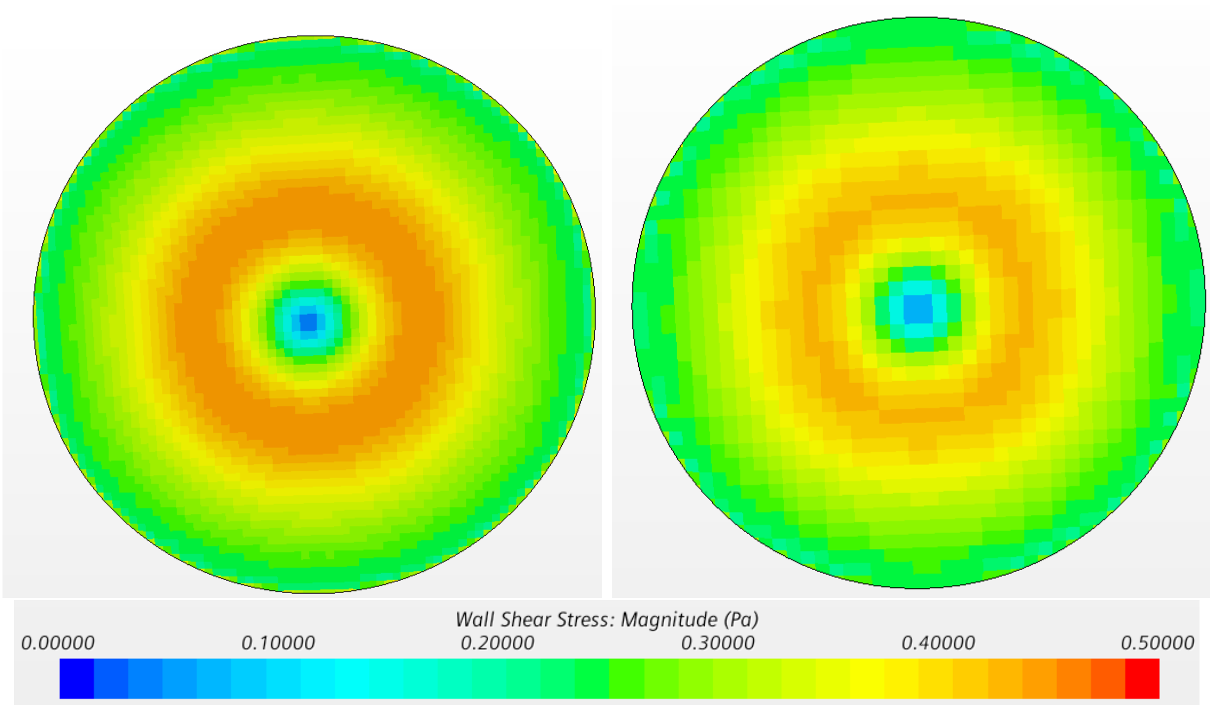


Figure 3.6: Apparatus shear stresses comparison. Refined mesh (left) and original mesh (right) for high shear configuration.

The four simulations are setup according to the above parameters and run until 1000 iterations are reached. At this point, all simulations have converged and are thus ready to be evaluated.

Chapter 4

Results

As described previously, we have considered four scenarios divided in two configurations – high shear and low shear – and two loading conditions – downward motion of the piston and upward motion of the piston. The shear stresses and relative pressure at the bone cell interface corresponding to each scenario are presented in the following.

I Configuration 1 - High Shear

I.1 Downward piston motion

Maximum shear stresses are obtained at the bone cell interface when the piston travels downward. Figures 4.1 and 4.2 show the results when the piston travels downward with velocity of 5mm/s.

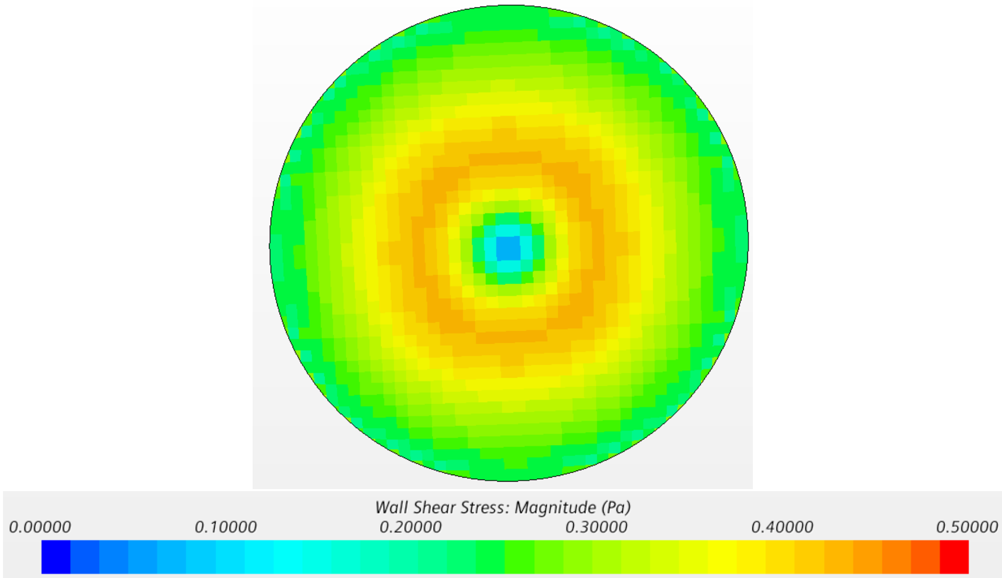


Figure 4.1: Sample shear stresses for high shear configuration and piston downward motion.

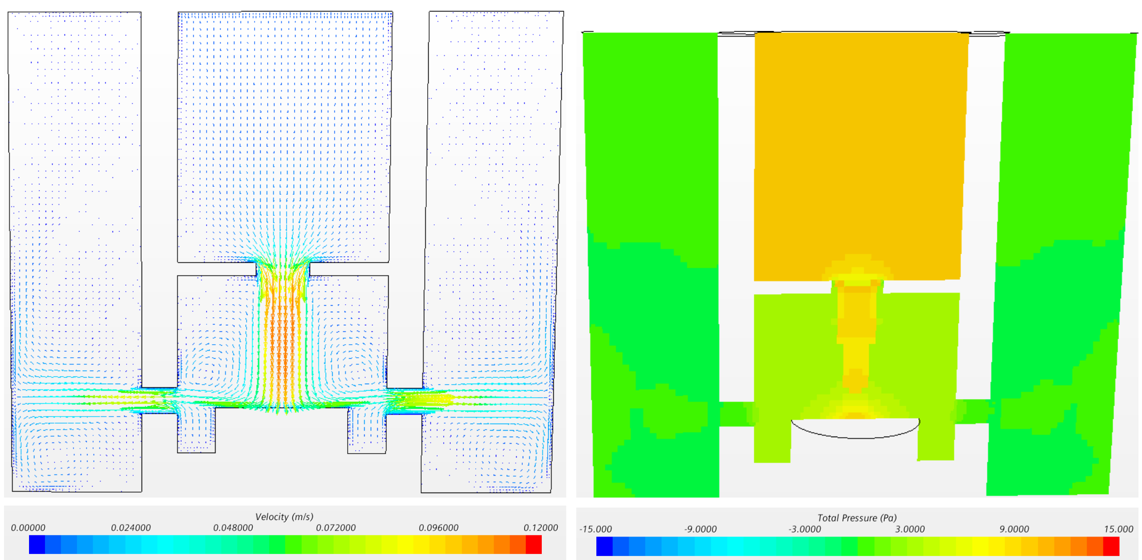


Figure 4.2: Apparatus flow (left) and relative pressure (right) for high shear configuration and piston downward motion.

I.2 Upward piston motion

The same configuration was then tested utilizing the same parameters except for a reversed flow direction, now representing the upward motion of the piston (Figures 4.3 and 4.4).

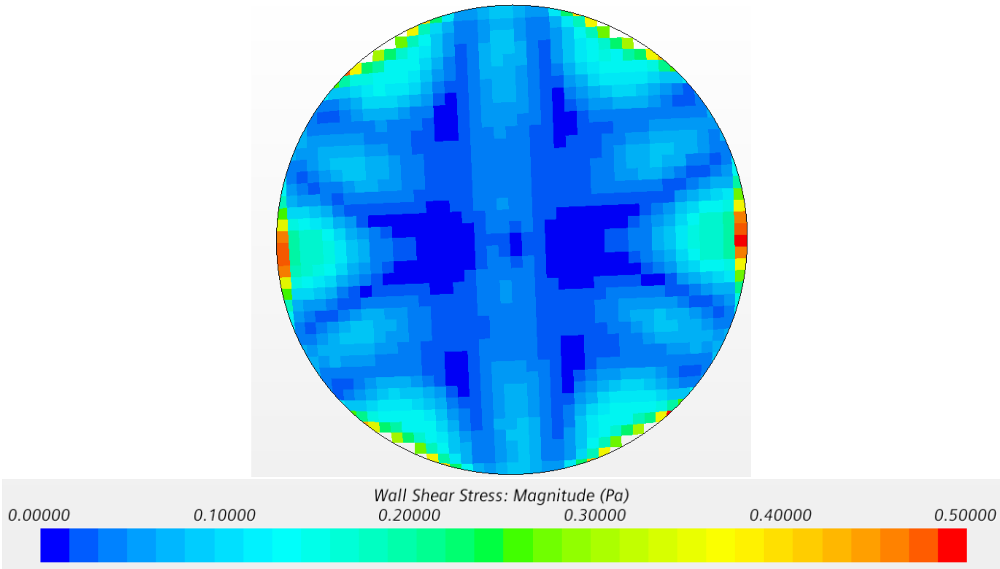


Figure 4.3: Shear stresses at the bone cell interface in the high shear configuration during the piston upward motion.

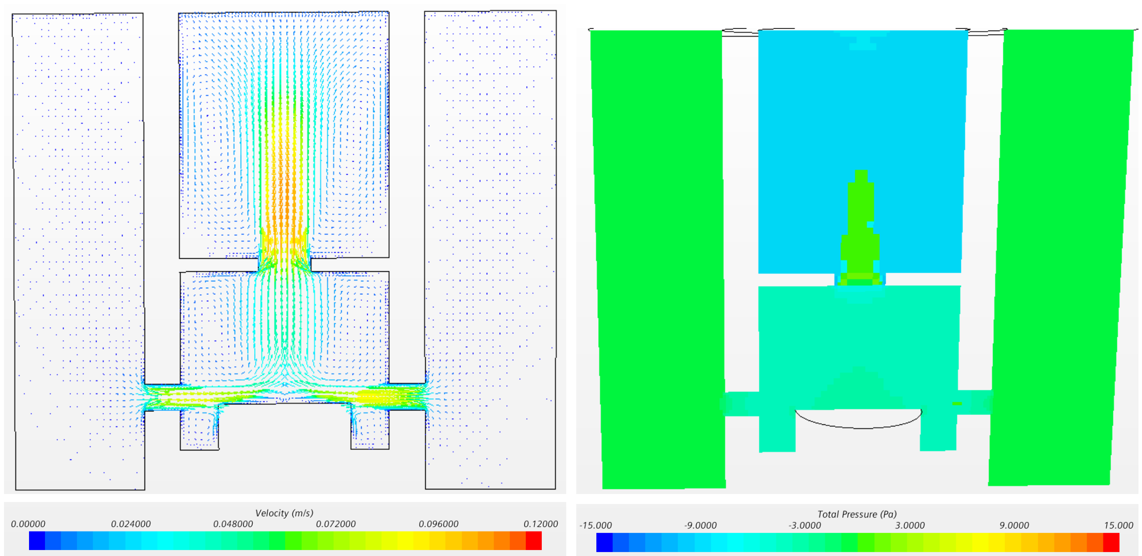


Figure 4.4: Apparatus flow (left) and relative pressures (right) for high shear configuration and piston upward motion.

II Configuration 2 - Low Shear

The low shear configuration was tested using the identical flow rates, meshing, and other analysis parameters.

II.1 Downward piston motion

Figures 4.5 and 4.6 show the the results when the piston travels downward with velocity of 5mm/s.

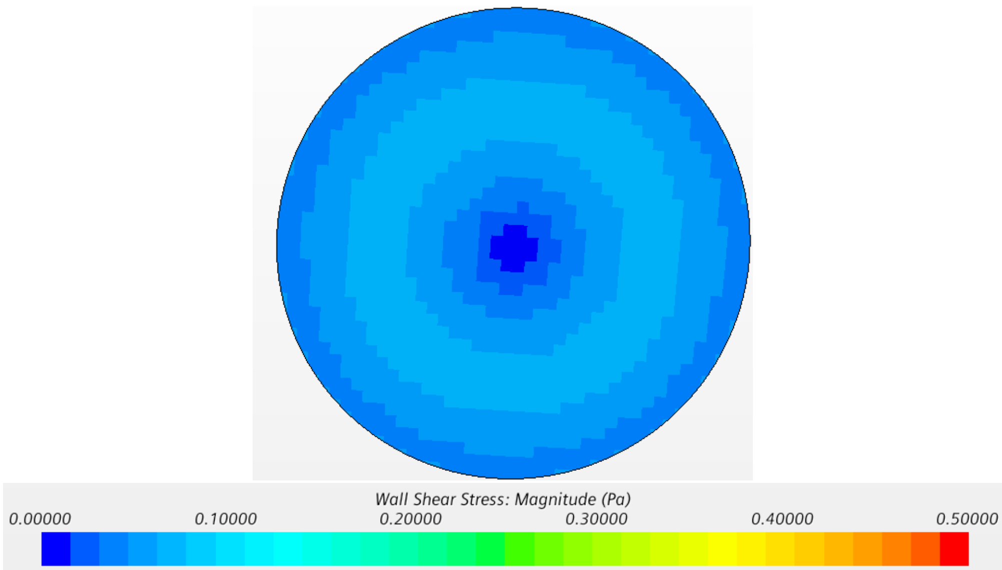


Figure 4.5: Sample shear stresses for low shear configuration and piston downward motion.

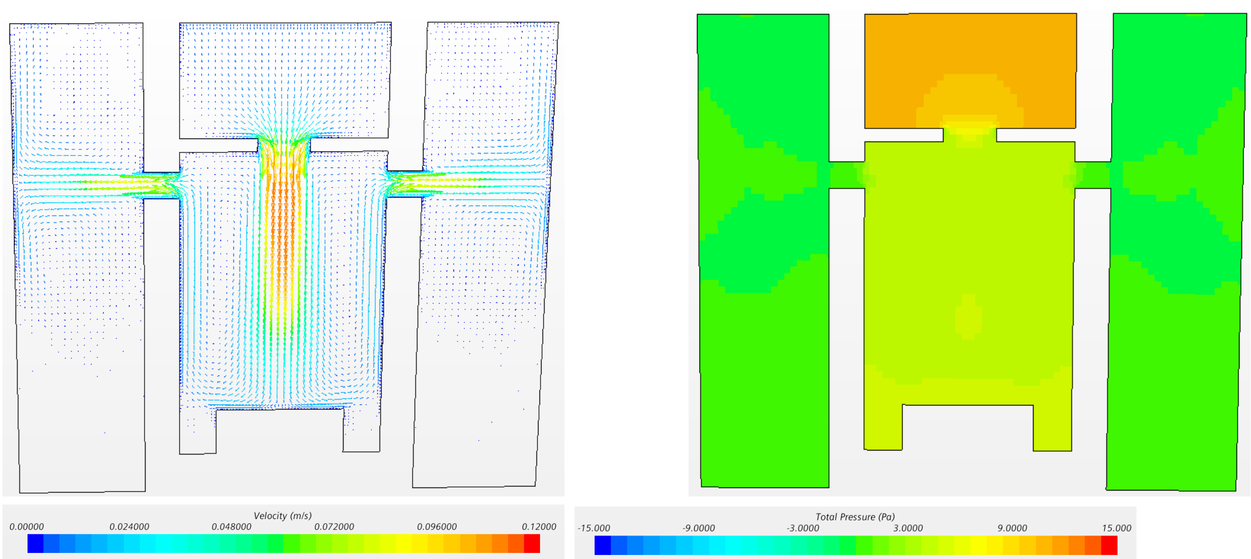


Figure 4.6: Apparatus flow (left) and relative pressure (right) for low shear configuration and piston downward motion.

II.2 Upward piston motion

The same low shear configuration was then tested utilizing the same parameters aside from a reversed flow direction representing the upward motion of the piston (Figures 4.7 and 4.8).

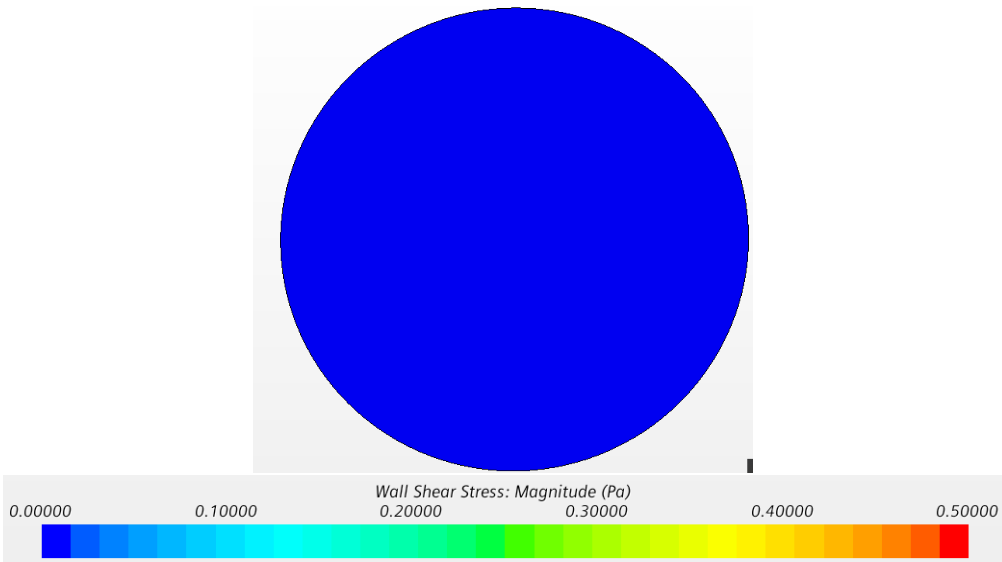


Figure 4.7: Shear stresses at the bone cell interface in the low shear configuration during the piston upward motion.

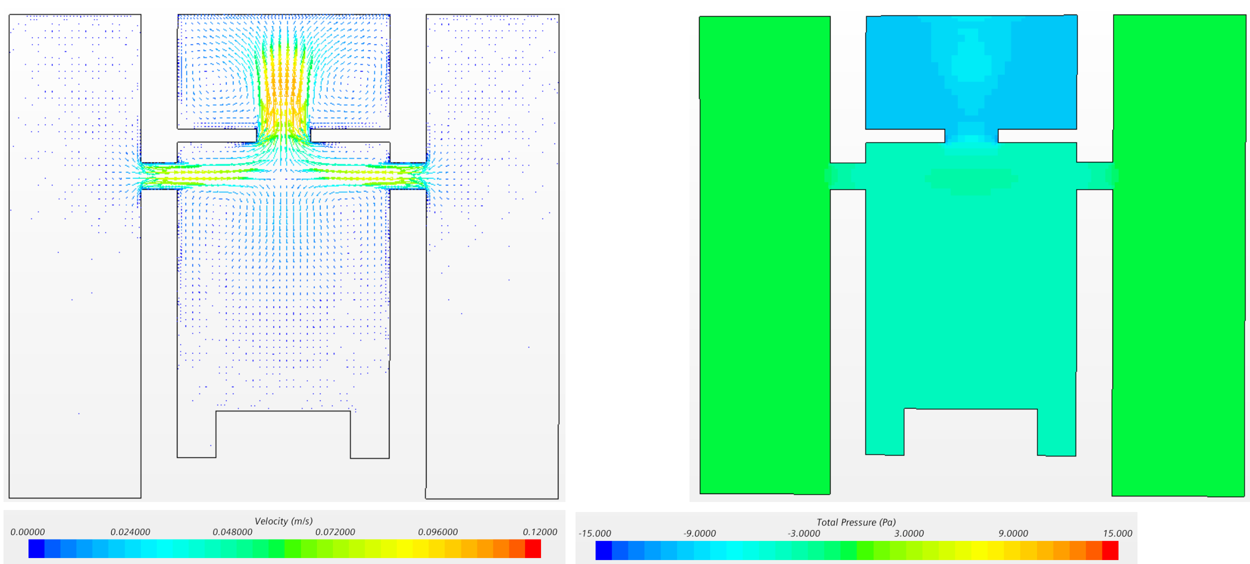


Figure 4.8: Apparatus flow (left) and relative pressures (right) for low shear configuration and piston upward motion.

III Summary of Results

The following tables report the approximate range of shear stresses (Table 4.9), relative pressures (Table 4.10), and flow velocities (Table 4.11) predicted by the low and high shear stress simulations.

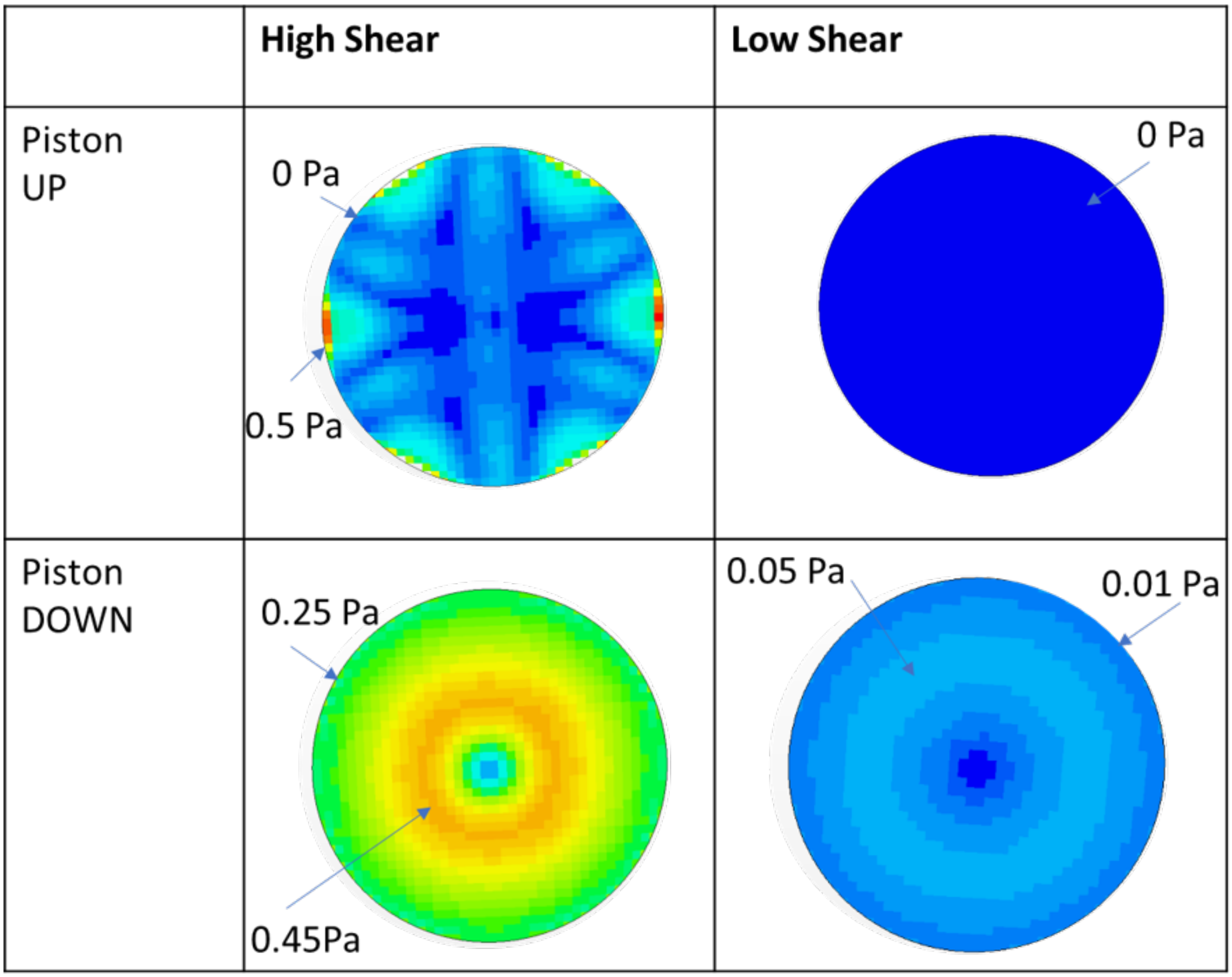


Figure 4.9: Summarized shear stresses at the bone interface.

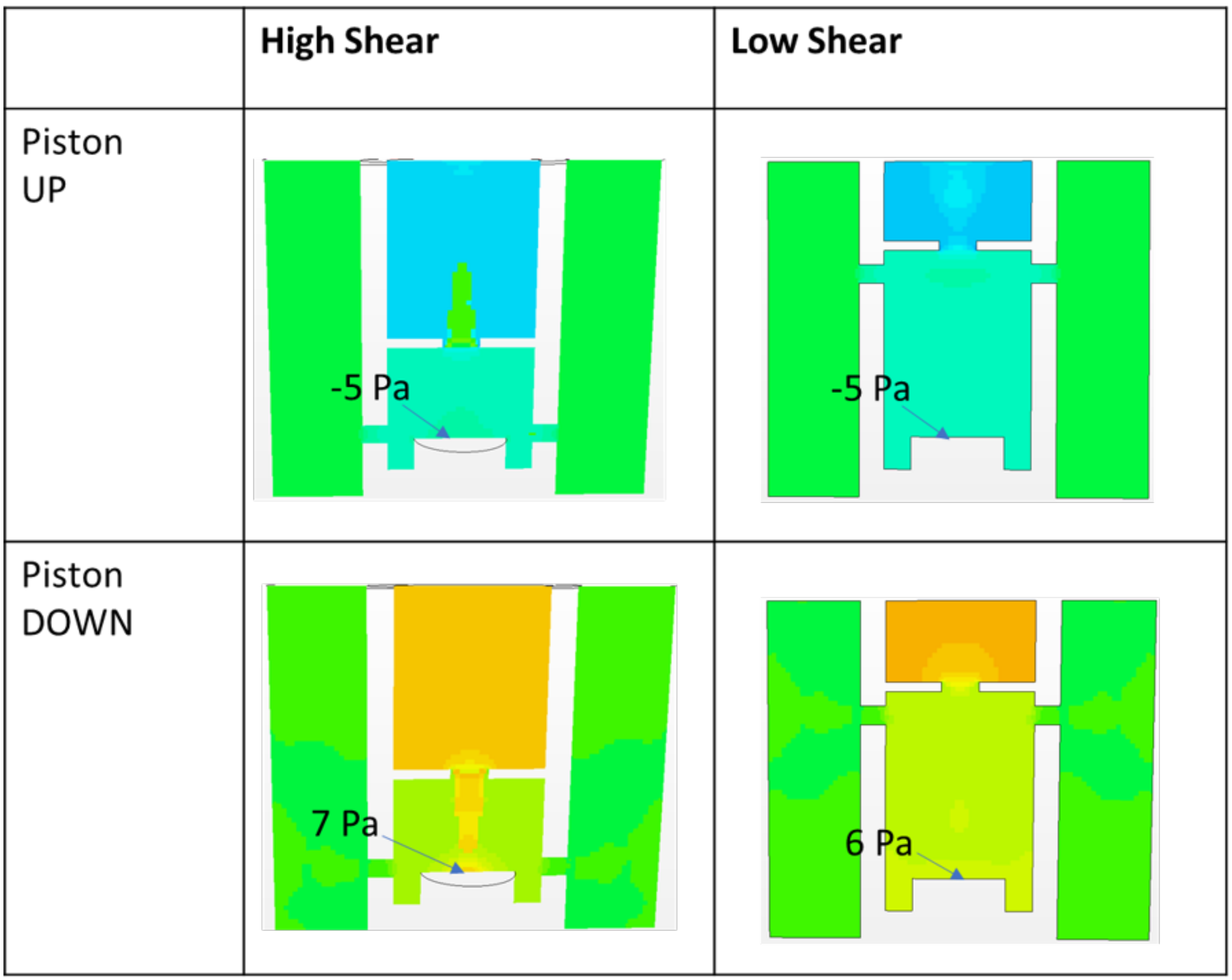


Figure 4.10: Summarized relative pressures at the bone interface.

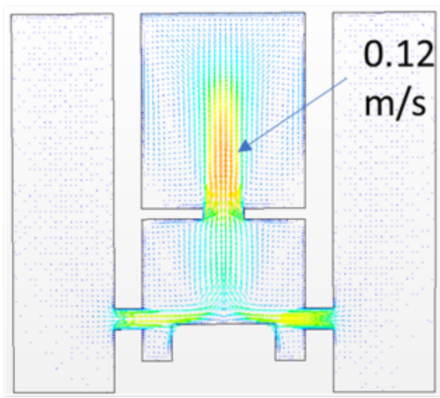
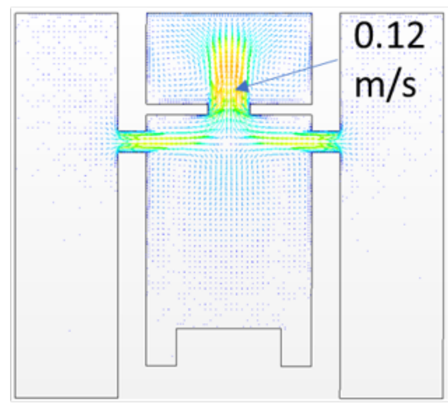
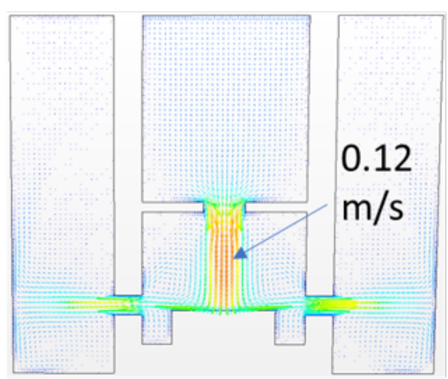
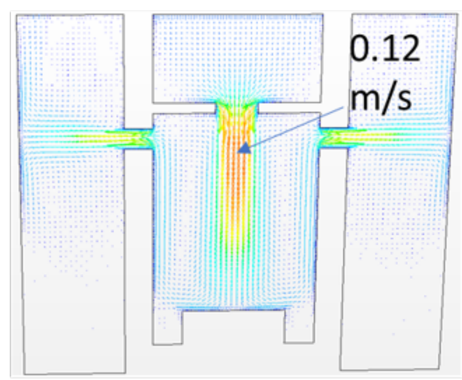
	High Shear	**Low Shear**
Piston UP		
Piston DOWN		

Figure 4.11: Summary of maximum speed at which the water travels after leaving the restricting nozzle.

Chapter 5

Conclusions

As expected the relative pressures at the bottom of the testing chamber are similar across configurations with average values between 7Pa and −5Pa for the downward and upward motion of the piston, respectively. The only notable difference between configurations consists in a slight relative pressure increase of 3Pa where the "jet" of water approaches the center of the sample in the high shear configuration. The magnitude of pressure expected to be experienced within the chamber significantly lower than what is required to stimulate bone growth on it's own [1][6] and such minute differences will likely have no meaningful impact on cell growth.

During the downwards piston motion a relatively uniform shear stress between 0.25Pa and 0.45Pa is obtained at the sample interface in the high shear configuration. In contrast, the shear stress in the low shear configuration is between 0Pa and 0.05Pa at the bone cell interface. When compared, shear stresses are approximately 5 to 10 times higher at any given location in the high shear configuration, with an average shear stress equal to approximately 0.35Pa compared to an average shear stress of approximately 0.025Pa in the low shear configuration.

Unfortunately, the upward piston motion is predicted to cause a less uniform shear stress pattern when compared to the downward motion. While this pattern may be useful to

investigate on it's own, the wide range of predicted shear stress may render the interpretation of the results more difficult.

While the non-uniform shear stress distribution during the piston motion is less than ideal, simple solutions do exist to mitigate this issue. Simply moving the piston upward at a slower speed would likely reduce surface shearing below significant values.

When focusing on the piston downward motion, the values predicted are consistent with the current literature and should be able to successfully stimulate bone cell growth [11, 14, 15]. The differences in fluid pressure across simulations appear to be negligible and bone cell growth, as intended, should be primarily stimulated by the shear stresses produced by flow motion in the apparatus.

Chapter 6

Future Work

In the future, the construction of the simulated device is to be completed along with an evaluation of the accuracy of the simulations through a load cell located at the bottom of the device. Of particular importance is the evaluation of the currently applied boundary conditions. Once validation has been completed, a growth media is to be determined and simulated. Subsequently, tests within an incubator can be conducted with the proposed apparatus and new findings may be made regarding the specific role that shear stresses play in bone development.

Chapter 7

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