Stress analysis in atherosclerotic arteries

The influence of heterogeneity

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Abstract

Atherosclerosis is a progressive arterial disease in which fatty tissue accumulates in the arterial wall. Due to this lipid uptake, the artery thickens and can eventually rupture, causing the lipids to flow into the bloodstream and form blood clots. If this happens in the carotid arteries, which supply the head with oxygenated blood, a stroke can be the result. If a rupture of an atherosclerotic site, called a plaque, occurs in a coronary artery, a heart attack can be the consequence. If a patient is diagnosed with a rupture prone plaque, several intravascular procedures can be performed to remove the rupture risk.

Since current clinical diagnostic tools are of limited value in predicting rupture risk, new methods are being developed. One of those methods is biomechanical modeling of plaques. With the use of Finite Element Analysis, the stresses in the plaque are being calculated and the stress levels are used as indicators of rupture risk.

Currently used biomechanical models assume homogeneous material distributions of the materials that are present in the plaque. However, especially the inner layer of the artery, called the intima, is far from homogeneous in atherosclerotic arteries. Since no previous studies included heterogeneity in plaque stress calculations, the first goal for this study was to create a method in which heterogeneity is included in plaque stress analyses. In stead of using Finite Element software in the conventional way, in which homogeneous sections are created, each individual element has its own material model. Hence, a completely heterogeneous distribution of material properties can be simulated.

To validate the method of individually assigning material properties to each element, a comparison between the conventional and the new approach is performed. Since the conventional approach is unable to include heterogeneity, the comparison was performed on homogeneous models. The resulting differences were significantly small enough to validate the new approach and the first research goal is met.

The second goal of this study is to determine the influence of heterogeneity on stress levels in the plaque. For this study, 12 images of plaques were available and for each plaque 100 different heterogeneous stiffness distributions were created. The distributions were based on grayscale values and on stiffness values reported in literature. Besides 100 heterogeneous calculations, also a homogeneous calculation per plaque was performed, to be able to compare the two approaches.

It was found that heterogeneity has a large effect on stresses in the plaque. The mean stress of the heterogeneous calculations was generally close to the homogeneous stress, the mean relative difference is $5 \pm 15.8\%$. However the variation that was found in the heterogeneous calculations was considerable in each plaque. Standard deviations, relative to the mean, ranged from $14\%$ up to $75\%$.

To increase the mechanical knowledge on plaque stress analyses, correlations between stiffness of different locations and stress levels were statistically determined. Although the setup of the study was not ideal for this task, some relations were found. It was found that the stiffness of a thin layer that separates the lipids from the lumen, called the cap, was most influential on the relevant stress levels in the plaque.

Since it is currently not efficient to perform a large number of heterogeneous calculations, it would be valuable to be able to predict the range that is introduced by adding heterogeneity to the equation. To accomplish this, correlations between geometrical properties and variation in stress levels were determined. Two relations were found. A larger lumen and a thinner cap lead to more variation.

It can be concluded that both goals were met in this study. Heterogeneity is successfully added to biomechanical modeling of plaques and it was found that heterogeneity has a strong influence on stress levels in the plaque.
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1. Introduction

Cardiovascular diseases are one of the leading causes of death in western societies. In the Netherlands 23% of deaths in 2012 was caused by a cardiovascular disease according to CBS (Dutch Central Bureau of Statistics) [1]. This makes it the second highest cause of death after cancer. Two of the most prominent consequences of cardiovascular complications are strokes and acute myocardial infarction, the latter also known as heart attacks, see figure 1.1. Both of these causes of death are usually a result of severe atherosclerosis.

In section 1.1 atherosclerosis will be introduced. The progression of the disease, the diagnostics and treatment will be discussed. Since currently used diagnostics are of limited value, new ways of determining whether or not to perform surgery are being developed. A promising approach is by using biomechanical modeling. This approach will be introduced in section 1.2. Although promising, biomechanical modeling is not yet developed into a robust tool. In section 1.3 will be discussed what this graduation project aims to contribute to the research of developing biomechanical modeling into a clinical diagnostic tool.

1.1 Atherosclerosis

Atherosclerosis is an arterial disease characterized by a local thickening of the arterial wall. This local thickening is mainly caused by an uptake of lipids from the blood. Atherosclerosis is a progressive disease that almost everybody develops over time. However, the level of atherosclerosis varies greatly from on person to another. In severe atherosclerosis the artery can eventually rupture, causing the lipids that are present in the atherosclerotic artery to flow into the blood stream. These lipids form blood clots that can completely obstruct the blood flow.

![Number of cardiovascular deaths for different causes and per gender in the Netherlands in 2010](image)

Source: CBS

Figure 1.1: Number of deaths of several cardiovascular diseases. Acute myocardial infarction (heart attack) and strokes are mainly caused by atherosclerosis.
To be able to prevent rupturing of atherosclerotic sites (called plaques), some background knowledge on arteries and on atherosclerosis is essential. This information will be given in this section.

1.1.1 Composition of arteries
Arteries consist of different layers with different functionalities. Due to atherosclerosis the composition of these layers changes.

**Healthy arteries**
A healthy artery is composed of three layers, see figure 1.2.A. The lumen, through which the blood flows, is surrounded by a layer called the intima. This single-cell layer is composed of endothelial cells, which are in direct contact with the bloodstream. These endothelial cells take care of the communication between the blood and the artery. The middle layer, the media, is made up of smooth muscle cells and elastic fibrous tissue. It is the most load bearing layer of the artery. The outermost layer of the artery is the adventitia. This layer is mainly composed of collagen and connects the artery with its surrounding tissue.

**Atherosclerotic arteries**
Libby [2] gives a thorough description of the formation and growth of atherosclerosis. Excess low density lipids (LDL, also known as bad cholesterol) is taken up in the vessel wall. This causes the endothelial cells of the intima to display adhesion molecules, which attract inflammatory cells like monocytes, and draw these into the intima. Within the intima, the monocytes transform into so called macrophages. These macrophages consume LDLs and over time become fat-laden, by this time they are called foam cells. The growth of these cells continues under the influence of inflammatory molecules. Figure 1.2.B shows a schematic view of an atherosclerotic artery. Figure 1.2.C shows a histology image of a real atherosclerotic artery. Due to a color staining, the different layers and the lipid core are visible.

From these images, it can be seen that the intima is the layer that is changed drastically with respect to the healthy intima. It transformed from a single-cell layer into a thick layer with different components in it.

1.1.2 Vulnerability of plaques
The lipid buildup and growth of the intima happens gradually over time. Stary et al. [3–5] define six stages of atherosclerosis, see figure 1.3. When the first lipid is detectable in an artery, it is called type I. In type II, the lipids are forming streaks. When the amount of lipid grows and small pools start to exist, the atherosclerosis transforms into type III. In type IV, called atheroma, the small pools of type III merge into one large pool. If a thick fibrous layer starts to develop over the lipid pool, the plaque is said to be of type V. Type V plaques,

![Figure 1.2: A) shows a schematic figure of a healthy artery, with indications to the intima, media and adventitia. B) gives a representation of an artery with atherosclerosis. It can be seen that the intima is transformed from a thin layer to a much thicker layer. C) gives an histology image of a real atherosclerotic artery. Due to color staining, different components of the artery are distinguishable. Source for A) and B): Biomechanical modeling of atherosclerotic plaques for risk assessment, dissertation of Ali Akyildiz. C) is a courtesy of Virmani.](image-url)
also known as fibroatheromas, can be subdivided into three different categories: type Va has a large lipid core, in type Vb the lipid core is mainly calcified and a plaque is called Vc if the lipid core is absent. If a plaque shows any sign of ruptures or hemorrhage, it is labeled as a type VI.

Type IV or Va atherosclerosis can cause an obstruction of the blood flow through the artery, which can result in a lack of oxygen being transported to the organs to which the artery is leading. The narrowing of the artery is called stenosis. This stenosis is not the only harmful symptom of atherosclerosis. If the amount of lipid increases and only a thin layer of fibrous tissue separates the lipid pool from the lumen, there is a risk of a rupture of this thin fibrous cap. The lipids can flow into the bloodstream and cause a blood clot, which can completely obstruct the blood flow. If this happens in a carotid artery, the blood clot will flow towards the brain. If it gets stuck in an artery in the brain, it can completely obstruct the blood flow to parts of the brain, which can lead to severe brain damage or even death.

1.1.3 Diagnostics & Treatment

To be able to prevent a plaque from causing severe health issues by rupturing, it is essential to know which plaques are vulnerable and which are not. Currently, the decision on whether or not to perform surgery on a carotid artery is based on the grade of stenosis. According to the Dutch guidelines of vascular surgery on strokes [6] women with a stenosis of over 70% and men with a stenosis of over 50% undergo surgery. If the stenosis is below these percentages, but the patient has an increased risk on developing atherosclerosis due to risk factors, medicines can be prescribed to fight the progression of atherosclerosis.

If the grade of stenosis is above the threshold, two types of surgery can be performed. The most frequently used surgery is called endarterectomy. In this procedure the artery is cut open and the plaque is surgically removed. Since the entire plaque is gone, the risk of health issues is very effectively reduced. The second surgery is called balloon angioplasty. In this minimally invasive procedure a catheter is threaded from the femoral artery (the artery that runs through the thigh) via the aorta to the carotid artery. At the site of the stenosis, a balloon is inflated to dilate the carotid artery and thus removing the stenosis. To enforce this enlargement, a stent can be placed. A stent is a wire-mesh tube that stays in the artery after the procedure.

These two procedures are very effective in removing the risk of plaque rupture and therefore removing the risk of a stroke. But currently, too many patients are undergoing the risk of surgery. Rothwell and Warlow [7] state in Lancet: "Surgery is needed in about 14 patients to prevent one having an ipsilateral carotid territory
major ischaemic stroke lasting longer than 7 days over the next 5 years.” If an improvement in diagnostics would be able to reduce the number of surgeries that is needed to prevent a single stroke, fewer patients would be subjected to the risk of surgery.

To improve this ratio of one in fourteen, a better way of defining a vulnerable plaque needs to be developed. Since the phenomenon that is trying to be prevented is the rupture of a plaque, not merely the grade of stenosis, the risk of actual rupture should be determined. The rupture of a cap is in fact a biomechanical process. If stresses in this cap exceed the strength of the material, it will rupture. Therefore, biomechanical modeling of stresses in atherosclerotic arteries can be used as a diagnostics procedure.

1.2 Biomechanical modeling

With biomechanical modeling of atherosclerotic arteries a numerical approach is used to estimate the stresses that are present in the artery. This numerical approach is Finite Element Analysis (FEA). A numerical model of the geometry of the plaque is created and the blood pressure is simulated. The stresses and deformations are calculated and are a measure of rupture risk. Especially the peak cap stress (the maximum stress in the cap area) is considered to have a high predictive value.

1.2.1 Finite Element Analysis

To be able to perform FEA on plaques, certain input information is necessary. The geometry of the plaque, the material properties of all components and natural and essential boundary conditions need to be defined.

The geometry is derived from medical imaging techniques, such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT) or Ultrasound Imaging. Based on one of these imaging techniques, a model is constructed in FEA software. This model consists of numerous small elements, called the mesh. Figure 1.4 gives an example of a mesh. This example shows a course mesh, so that individual elements are easily visible. By reducing the mesh size, the accuracy of the FEA increases.

In current FEA on plaques, each layer or component of the artery gets a material model assigned to it. These material models describe the relation between stresses and deformations. Currently, all layers and components are modeled as homogeneous materials. Some studies do include anisotropy of fibers in their models.

![Figure 1.4: An example of a mesh of a plaque is shown. This mesh is very course, so that individual elements are visible. Different components in the artery have different colors.](image-url)
The natural boundary condition in FEA on plaques is the blood pressure. By applying a uniform pressure on the lumen border, this blood pressure is mimicked. Essential boundary conditions need to be added to prevent unnecessary translation and rotation of the model when solving the model for stresses and deformations.

1.2.2 Heterogeneity in plaque modeling

In a literature research performed for this graduation project [8], it was found that the material behavior of the atherosclerotic intima is not homogeneously distributed. A high level of heterogeneity within the intima was found. Chai et al. [9] reported Young’s moduli ranging from 6 up to 891 kPa for the carotid intima. In their study, μ-indentation tests were performed to measure local material behavior.

Although literature shows the heterogeneous nature of the intima, this heterogeneity is not yet included in FEA on plaques. It is therefore unknown whether or not heterogeneity of the intima has a significant and relevant influence on the stresses in the plaque.

1.3 Research goal

This graduation project focuses on heterogeneity in the atherosclerotic intima in FEA. Two main goals are set:

The first goal is to create a method that allows to model heterogeneity in the intima.
The second goal is to find the influence of intima heterogeneity on stresses in the artery.

1.3.1 Approach

To introduce heterogeneity in the intima, FE software needs to be used in a different way than with conventional methods. With conventional methods, the entire intima is seen as a single section with a single material model, leading to a homogeneous intima. To be able to add heterogeneity, the intima can not be seen as a single section. Each element of the mesh is seen as a single section in this study. Each element has its own material model and therefore, the material behavior can vary per element.

Material properties need to be assigned to elements in a sensible way. In this study four clusters in the intima are defined, based on a histology images of the modeled plaque. Each cluster will get its own stiffness properties, leading to a stiffness map of the entire artery. Based on this stiffness map material properties are assigned to all elements of the FE model and a heterogeneous FE model is born.

To determine the influence of heterogeneity on stress levels in the plaque, for 12 plaques 100 simulations with different stiffness distributions are performed. The resulting stresses of these simulations are compared to homogeneous calculations for the same 12 plaques.

In chapter 2 the heterogeneous method is discussed in detail. In this chapter it is also explained which analyses are performed to validate the new methodology and to determine the influence of heterogeneity on stress results. The results of these analyses are reported in chapter 3. A discussion and conclusions on the findings of this study are presented in chapter 4.
2. Methods

In this study, a new approach is developed to use 2D Finite Element Analysis (FEA) for atherosclerotic arteries. The main difference with previous approaches is that heterogeneity is included within the intima. Furthermore, no distinction in the FE mesh is made between the different layers of the artery. In stead of segmenting components and creating a mesh based on this segmentation, a segmentation-less FE mesh is created. Material properties are assigned element-wise, based on imaging data. This allows for heterogeneity in material properties.

Several steps need to be taken to successfully implement heterogeneity in FEA. First of all, a stiffness map of the plaque needs to be created. Section 2.1 will explain how this stiffness map is made, based on the histology of a plaque. Based on this stiffness map, FEA is performed to calculate the stresses in the plaque. Section 2.2 will explain how a heterogeneous FE model is created and how the stresses in the plaque are calculated.

To determine the influence of heterogeneity, statistical analyses are performed with the newly developed tool. These are discussed in section 2.3. First of all, a validation of the segmentation-less approach is explained. In this section it is also explained how the heterogeneous approach is compared to the homogeneous approach. Furthermore, the relations between input and output parameters are discussed, to find the influence of heterogeneity on stress levels.

2.1 Heterogeneous stiffness maps

Based on the histology of a plaque a stiffness distribution will be created in MATLAB. In this distribution, different layers and components are represented: the media/adventitia layer (arterial wall), the intima and the lipid core. These components are manually segmented. Heterogeneity in the intima is introduced, by creating multiple clusters within the intima, based on the grayscale values of the histology. By giving each layer and cluster its own stiffness, a distribution is created which includes the different layers and components and a heterogeneous intima. More details will be given below.

2.1.1 Manual segmentation

The first step in creating a heterogeneous stiffness map in MATLAB is to manually segment the different layers and component in the plaque. Although the media and adventitia are different layers, they are often

Figure 2.1: On the left-hand side the histology of a plaque is shown. On the right-hand side, the manual segmentation is shown. The outer layer is the wall, which consists of the media and adventitia layer. The light-blue layer is the intima and the dark-blue component is the lipid core.
treated as a single layer in FEA. Therefore, in segmenting the different layers, these two are seen as a single layer: the wall. The wall, the intima and the lipid core are delineated, based on the histology of the plaque. Figure 2.1 gives an example of a manual segmentation of a plaque.

2.1.2 Automatic clustering

Akyildiz et al. [10] state that there are four components dominant within the intima of an atherosclerotic artery: smooth muscle cells, collagen, extracellular matrix and calcifications. Unfortunately, it is nearly impossible to delineate these components in the histology images that are available for this study. It is however possible to distinguish clusters based on grayscale values. These clusters most certainly do not have a one-on-one relation with components in the intima, but the clusters do have realistic shapes and sizes that are representable for structural components in the intima. Figure 2.2 shows an example of a clustering result of a plaque.

The clustering is done based on a k-means algorithm. This algorithm iteratively searches an optimal cluster distribution, based on grayscale values of pixels in the intima. Location of the pixels is completely neglected. Grayscale values vary from 0 to 255. Four starting points are chosen for the algorithm at random in the range of 0 to 255. All pixels in the intima are assigned to the the starting point with the closest value. This leads to four clusters. The mean grayscale value of each cluster is a new starting point for the next iteration. After each iteration, four new starting points are determined. Again, all pixels are assigned to the new starting point which is closest to its own grayscale value. If the clustering no longer changes from one iteration to the next, the algorithm has found an optimal cluster distribution. Optimal is in this case defined as the minimum of the sum of squared differences of all grayscale values to the mean of their assigned cluster, see equation 2.1.

\[
\min \sum_{i=1}^{4} \sum_{n=1}^{N_i} (x_n - \mu_i)^2
\]  

(2.1)

In this equation, \(N_i\) is the number of pixels in cluster \(i\), \(x_n\) is the grayscale value of pixel \(n\) and \(\mu_i\) is the mean grayscale value of cluster \(i\). The four clusters can each have a different cluster size. Figure 2.3 gives an example of the k-means clustering. The graph shows the grayscale values of all pixels in the intima and the cutoff values for the four clusters.

The k-means clustering assigns all pixels to one of the four clusters. Since the k-means algorithm does not take pixel location into account, isolated pixels can exist. Since the clusters are supposed to mimic size and shape of different structures, these isolated pixels should not be present. Therefore an erosion and reconstruction step are made. These steps remove isolated pixels and are therefore essential to mimic biological structures.
2.1.3 Stiffness assignment & Smoothing

With the manual segmentation and automatic clustering, six clusters are created: the arterial wall, the lipid core and four clusters that span the entire intima. Each of these six clusters will get their own stiffness assigned to it, based on values found in literature. The wall will get a stiffness of 1200 kPa and the lipid core 5 kPa. The stiffness of the four clusters in the intima will vary from 6 up to 891 kPa. The values for the intima are the extreme values of a range of stiffness values found by Chai et al. [9]. Since the segmentation and clustering are performed on the histology image, the stiffness matrix that is created has the same size as the pixel size of the histology image. In figure 2.4 the left-hand side image gives an example of a stiffness distribution. It can be seen that there are discontinuities along the edges of each clusters. These discontinuities will lead to numerical errors when performing FEA.

To remove the discontinuities, a 2D Gaussian filter is used to create a gradient between the different stiffnesses. Conventional filters in MATLAB can not deal with the lumen border and the outer border of the vessel. Therefore a new filter function has been designed. A Gaussian kernel moves over the entire non-smoothed stiffness map and only values that are located in the artery are used to smoothen the stiffness map. On the right-hand side of figure 2.4 the stiffness map has been smoothed with a Gaussian filter with a standard deviation of 5 pixels. Some smoothing is essential, but with too much smoothing, the shapes of the clusters are lost.

2.2 Finite Element Analysis

To be able to actually perform stress analyses on the generated stiffness maps, FEA is used. FEA calculates stresses in a structure based on the geometry, material properties and boundary conditions. Since no studies have included heterogeneity in FEA of atherosclerotic arteries, a method has been developed that implements the stiffness maps that are created in MATLAB. In this study ABAQUS has been used as FEA software.

2.2.1 Heterogeneous FEA for atherosclerotic arteries

Current methods to perform FEA on atherosclerotic arteries all have a homogeneous approach. Based on the manual segmentation, different homogeneous sections are created in the FEA software. The next step is to generate a mesh. This mesh is dependent on the segmentation. An example of a homogeneous model with a segmentation-dependent mesh is shown in figure 2.5. In this example there are three sections: the wall, the intima and the lipid core. It can be seen that each section is homogeneous and that there are discontinuities in material properties present at the borders of each section.

The first step into creating a heterogeneous FE model is to create a simple model in ABAQUS, only based on the lumen border and the outside border of the artery. The entire artery is at this point treated as a single
Figure 2.4: On the left-hand side a stiffness map is shown that is created based on the manual segmentation and the automatic clustering procedures. To remove the discontinuities that are present on the left-hand side, Gaussian smoothing is applied. A stiffness map, smoothed with a Gaussian kernel with a standard deviation of 5 pixels is shown. In the close-ups, the difference between the non-smoothed and smoothed stiffness maps can clearly be seen.

section and a mesh is created. The difference between this new mesh and a mesh of the conventional method is that this new mesh is not dependent on any segmentation.

The stiffness for each element in the FE mesh is individually determined, based on the obtained stiffness map (section 2.1). To be able to do this, there are as many sections as there are elements. Each section contains a single element and has an individual material model. A MATLAB script is written to automatically assign sections and material properties to each element. Figure 2.6 gives an example of a heterogeneous distribution.

Material models

In this study, Neo Hookean material models are used. These hyperelastic models allow for a limited degree of non-linearity and are described by a strain-energy function (equation 2.2).

\[ W = c_{10} (I_1 - 3) + \frac{1}{D_1} (J^{el} - 1)^2 \]  
\[ I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \]  
\[ J^{el} = \lambda_1 \lambda_2 \lambda_3 \]  
\[ c_{10} = \mu/2 = E/6 \]  
\[ D_1 = \frac{2}{\kappa} \]  

In these equations, \( c_{10} \) and \( D_1 \) are material parameters and \( I_1 \) and \( J^{el} \) are respectively the first invariant and the determinant of the Left-Cauchy-Green deformation tensor. \( I_1 \) and \( J^{el} \) (equation 2.3,2.4) are dependent on stretch ratios \( \lambda_i \) and are therefore representatives of the state of the material. The relation between parameter \( c_{10} \), shear modulus \( \mu \) and Young’s modulus \( E \) is given in equation 2.5. Parameter \( c_{10} \) is an indicator of the stiffness of the material. \( D_1 \) is dependent on bulk modulus \( \kappa \) and represents the compressibility of the material. In this study, \( D_1 \) is always chosen at \( 10^{-5} \), which makes all materials nearly incompressible.

2.2.2 Boundary conditions

Several boundary conditions need to be applied to calculate the stresses in a plaque model. Since the geometry was deduced from histology images of arteries that were fixed at 100 mmHg, the original geometry is not stress-free. Therefore, initial stresses need to be calculated. After these initial stresses are known, the systolic blood pressure is applied. To prevent the model from rotating and or translating, essential boundary conditions are applied. Figure 2.7 shows the boundary conditions.
Figure 2.5: An FE model with color-coded stiffness according to the conventional method is shown. Three sections are created, each with its own homogeneous material model. It can be seen that there are discontinuities along the borders of each section. In the close-up, it can be seen that the elements at the border of the sections are arranged in such a way that a fluent section border exists. This is due to the segmentation-dependent mesh.

Figure 2.6: An FE model with color-coded stiffness according to the heterogeneous method is shown. In this model, there are as many sections as there are elements and material models. Due to the smoothing that was applied on the stiffness map, no discontinuities are present. In the close-up, it can be seen that the elements are not aligned with the borders of the original segmentation.
To calculate the initial stresses, a Backwards Incremental Method (BIM) introduced by Speelman et al. [11] is used. The BIM takes the original geometry and step-by-step increases the pressure on the lumen border up to 100 mmHg (13.33 kPa). After each step, the stress in each element is calculated and the geometry is slightly deformed. This deformation is discarded and the geometry is reset to its original form and the calculated stresses are added to the model as initial stresses. For this study, 6 equal pressure increments were used to reach 100 mmHg.

After the initial stresses are calculated, the systolic pressure can be applied to the model. In this study a pressure of 120 mmHg is used as systolic pressure. 120 mmHg equals 16 kPa. After this loading condition has been applied, the stresses and strains that are present in the artery are determined.

To prevent the model from showing unwanted translations and rotations that do not influence stresses, essential boundary conditions are applied. These boundary conditions should not induce any stresses. In this study, a point on the lumen border is fixed in both horizontal and vertical direction and a point on the outer border is only fixed in horizontal direction.

2.3 Analyses

Since the segmentation-less mesh is a novel approach, a validation of the methodology is performed. Besides developing a new method to calculate stresses in a plaque based on heterogeneous material properties, this method is also used to determine the influence of heterogeneity on stresses in the plaque. The simulations that are performed to calculate stresses of heterogeneous plaques are explained in this section. The statistics to quantify the influence of heterogeneity on stress levels is also explained.

2.3.1 Validation of methodology

The methodology that is developed for this study contains four new components in FEA on atherosclerotic arteries. First of all, a mesh is used that is not dependent on the segmentation. Secondly, each element of the FE model has its own section and material model. Thirdly, a smoothing is applied on the material properties of the entire artery to eliminate discontinuities. And fourthly, heterogeneity is added to the intima.

![Figure 2.7: The orange triangles show the essential boundary conditions. A point at the lumen border is fixed horizontally and vertically and a point at the outer border of the artery is only fixed in horizontal direction. The black arrows show the blood pressure on the lumen border.](image)
Since heterogeneity has never been included in previous studies, there is no way of validating the end results of the new method. However, the segmentation-independent mesh can be compared with the segmentation-dependent mesh. Two homogeneous models are created to validate the segmentation-less approach:

- A conventional FE model with a segmentation-dependent mesh
- An FE model with a segmentation-less mesh with smoothing on material properties

For each model, a mesh refinement analyses was performed. An extremely fine mesh with over 60,000 elements was created and stresses were calculated. This simulation is considered to be the baseline. Courser meshes, ranging from 5,000 to 25,000 elements were created and again, stresses were calculated. If the difference in peak cap stress between a course mesh and the baseline is under 1%, the mesh size is small enough. For all models, the stiffness of the wall, intima and lipid core are the same (respectively 1200; 448.5 and 30 kPa), so the resulting stress levels can be compared.

### 2.3.2 Simulations with a heterogeneous intima

When performing FEA on plaques, the loading parameters and the material parameters need to be chosen. The loading is set at 120 mmHg (16 kPa). The Young’s moduli of the wall and lipid core are respectively 1200 and 30 kPa. In this study, the influence of heterogeneity of the intima is being investigated. Therefore, the Young’s moduli of the intima are not fixed, but are varied from 6 up to 891 kPa. All stiffness values are based on a study of Chai et al. [9] in which local stiffnesses in atherosclerotic arteries were determined by performing \( \mu \)-indentation tests.

Twelve histology images are used to create twelve different FE models, each with four clusters in the intima. Since an entire range of possible heterogeneous maps is to be tested, 100 sets of stiffness combinations for the four clusters of the intima are created. These 100 sets of four stiffness values are created by doing a Latin Hypercube Sampling (LHS) in MATLAB with a build-in function (lhsdesign).

In this particular case, the LHS gives a 100 x 4 matrix. Each of the 100 rows represents the four stiffness values of the four clusters for one of the 100 simulations of a plaque. Each column represents all the stiffness values that will be assigned to a particular cluster for each of the 100 simulations. For each column, 100 equally sized intervals between 6 and 891 kPa are created and a random value in each interval is picked. The picked values are placed into the column in a random order. For twelve plaques, twelve LHS’s of 100 x 4 are created. This leads to a total of 1200 heterogeneous simulations.

To be able to compare the heterogeneous to the homogeneous approach, for each plaque a homogeneous calculation was also performed. The same FEA method was used for the homogeneous approach, but all four clusters in the intima were set to the mean of the stiffness range: \( \frac{6 + 891}{2} = 448.5 \), kPa.

The most important value when performing FEA on plaques, is the peak cap stress. This is the highest stress that is present in the cap of the plaque and may indicate the likeliness of cap rupture. Since numerical errors can lead to single elements with an unusually high stress, this study uses the 95th percentile cap stress (95pCS) as an output parameter. The type of stresses that are used are maximum principle stresses.

Another value that is used is the amount of lumen change between the original and the inflated geometry due to the systolic blood pressure. Since an entire range of stiffness values is used, there is also a variation in lumen change. Relatively high and low lumen changes are not realistic and not comparable to the homogeneous simulation. Therefore the calculations with upper and lower 10% of lumen area change are discarded from the analyses. Figure 2.8 gives an example of the lumen changes for the 100 calculations of one plaque and shows the cut-off values at which calculations are discarded. Out of the original 100 calculations per plaque, 80 will be used for statistical analyses.

The intima of a plaque can be divided into multiple geometrical regions. The cap is the part of the intima that separates the lipid core from the lumen. The shoulder areas are the areas on both sides of the lipid core. The area behind the lipid core (the back) and the remainder of the intima are the other two regions. Figure 2.9 shows the different geometrical regions of a plaque. For this study, the shoulders are defined as 15° angles on either side of the lipid core. The four geometric regions are automatically determined by a MATLAB script, specifically written for this study. These regions are defined, so that correlations between the stiffness per region and 95pCS can be calculated.
Figure 2.8: This figure gives an example of the lumen change of 100 calculations of a plaque. The calculations are sorted by lumen change. The lower and upper 10% are discarded for analyses. These 10% areas are shown in gray.

Geometrical properties of each plaque are also determined. This study determined six different geometrical parameters:

- Minimum cap thickness
- Maximum lipid core thickness
- Lipid core angle
- Mean lumen radius
- Maximum lumen curvature
- Maximum lipid core curvature

Minimum cap thickness is defined as the minimal distance between the lipid core and the lumen border. Lipid core thickness is the minimum distance from the front-side to the back-side of the lipid core, so the maximum lipid core thickness is the maximum of all thicknesses. The lipid core angle is the angle that the lipid core makes, as seen from the center of the lumen, see figure 2.9. The lumen radius is the mean radius of the lumen.

Figure 2.9: This image shows automatically generated geometric regions. The cap is shown in light-blue, the shoulders in orange, the back of the lipid core in green and the remainder of the intima in yellow.
2.3.3 Analyses

Analyses were performed to evaluate the results of adding heterogeneity to the atherosclerotic intima on stress levels in the plaque. These analyses give a measure of the influence of heterogeneity and give insight in important parameters on stress levels.

An important goal of this study is to determine the influence of heterogeneity on 95pCS. To determine this influence, for each of the 12 plaques a boxplot is created. This boxplot contains the 95pCS for the 80 calculations that are evaluated. The boxplot gives a measure of uncertainty of 95pCS due to heterogeneity. To compare the heterogeneous 95pCS values with the homogeneous 95pCS, the homogeneous 95pCSs are shown in the boxplot as well.

Since a range of 95pCSs is found for each plaque with the heterogeneous approach, it is valuable to be able to predict the variation based on geometrical properties. Knowledge on the variation will increase the predictive value of the homogeneous approach by giving an uncertainty range. For six geometrical properties correlations with 95pCS-variation will be calculated. These correlations will be expressed in Pearson correlation coefficients.

Since there are four clusters defined in the intima, knowledge on which of these clusters is most influential on 95pCS can lead to an understanding of important locations in the intima. For each of the four clusters the Pearson correlation coefficient between the 95pCS and the stiffness of each cluster will be determined. Since all plaques have an individual clustering, the correlation coefficients are individually determined for each plaque.

Not only is the Pearson correlation coefficient with 95pCS determined for the stiffness of the clusters in the intima, it is also evaluated for the stiffness of the four geometrical regions. Each of these regions (cap, shoulders, back and remainder) can have a different influence on the 95pCS. A correlation coefficient will be determined for each individual region and individual plaque.

For two plaques the effect of changing stiffness of only a single cluster is evaluated. The other three clusters were set to the mean intima stiffness. For one plaque, the effect of a different clustering was investigated.
3. Results

In this study a new FEA approach is successfully developed. Heterogeneity is added to the atherosclerotic intima. Section 3.1 gives two examples of FE models, created with this new approach. Since the creation of these models is dependent on the clustering, the variation in cluster sizes is given. To validate the new mesh approach that is taken in this study, a comparison is made between the conventional and segmentation-less mesh approach. The results are given in section 3.2. To determine the influence of heterogeneity on stress levels in the plaque, several analyses were performed. The heterogeneous approach is compared to the homogeneous approach and correlations between input and output parameters are determined in section 3.3.

3.1 Generating heterogeneous FE models

For each of the twelve histology images a manual segmentation of the arterial wall, intima and lipid core was performed based on a previous segmentation, performed by an experienced user. The automatic clustering of the four clusters in the intima is successfully performed. Figure 3.1 shows two examples of the creation of a heterogeneous FE models. On the left-hand side, a histology image is shown. The middle images show the clusters that are automatically determined with the k-means algorithm. After assigning stiffnesses to the

Figure 3.1: For two different plaques the histology is shown on the left-hand side. The two images in the middle show the clustering of the intima and the right-hand side images give the FE models with on of the heterogeneous material distributions.
clusters and applying smoothing, a FE model is created. Two examples are shown on the right-hand side of figure 3.1. In appendix A all histology images and their clustering are shown.

The cluster sizes of each of the 12 plaques were different. The smallest clusters range from 0.08% of the intima up to 16.3%. The largest clusters for each plaque are in the range of 31.2% to 58.6%. The mean (± std) cluster size is 25 (± 15.4)%.

3.2 Validation of methodology

Figure 3.2 shows the two FE approaches that were used for the validation. On the left-hand side is the conventional method with a segmentation-dependent mesh. In the FEA software, the contours of the segmentation are drawn and a mesh is made, based on these contours. The right-hand side image gives an FE model with a segmentation-independent mesh and with smoothing applied to the material properties. This mesh is created without drawing the contours. The material properties of the FE model are obtained from a stiffness map (section 2.1). The top row of images shows color-coded Young’s moduli and the bottom row gives the stress results of the models. Both methods show peak stresses at an equal location on the lumen border. The general stress distribution is very similar. However, the local peak stress at the lipid core boundary that shows up on the conventional method does not show up on the segmentation-less method.

For both methods, a different mesh size has been tested. For each model, a mesh with approximately 5,000; 10,000; 15,000; 20,000; 25,000 and 60,000 elements have been created. The 95pCS is used to evaluate the results. Figure 3.3 shows the 95pCS for both models and all simulations. At 20,000 elements, both methods
are within 1% error relative to their 95pCS with 60,000 elements. Therefore, approximately 20,000 elements are used for further analyses.

At 20,000 elements, the 95pCS of the conventional method is 86.1 kPa. The 95pCS of the new method is 83.2 kPa. The relative difference is 3.5%.

3.3 The influence of heterogeneity on 95pCS

Homogeneity vs Heterogeneity

Figure 3.4 shows the results for all twelve plaques. The boxplots show the median, interquartile and total range of 80 calculations per plaque. The whiskers of the boxplots have a maximum length of 1.5 times the length of the interquartile range. All points that are further away are considered to be outliers. In this boxplot the homogeneous 95pCSs are shown with the black dots. Homogeneous values were calculated with the same methodology as the heterogeneous ones, but all clusters were set to the average intima stiffness (448.5 kPa).

The mean 95pCS of heterogeneous calculations per plaque ranges from 15.9 to 88.6 kPa. Standard deviations range from 4.1 to 17.8 kPa. Standard deviations relative to their mean range from 13.6% to 74.5%. The mean 95pCS of heterogeneous calculations is compared to homogeneous 95pCS. The mean (± std) difference is 5.8 (± 15.8)%.

All values are given in table 3.1.
Table 3.1: This table gives an overview of the results of all performed simulations. For each of the 12 plaques, the homogeneous 95pCS is shown and on the right side of the dashed line, the heterogeneous 95pCS for the plaques is represented by the mean, standard deviation in kPa and in % of the mean and the minimum and maximum values.

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Homogen. (kPa)</th>
<th>mean (kPa)</th>
<th>std (kPa)</th>
<th>std (%)</th>
<th>min (kPa)</th>
<th>max (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33.74</td>
<td>44.68</td>
<td>8.37</td>
<td>19</td>
<td>32.31</td>
<td>73.57</td>
</tr>
<tr>
<td>2</td>
<td>45.78</td>
<td>47.89</td>
<td>17.77</td>
<td>37</td>
<td>18.41</td>
<td>92.79</td>
</tr>
<tr>
<td>3</td>
<td>19.03</td>
<td>17.42</td>
<td>10.39</td>
<td>60</td>
<td>-0.25</td>
<td>58.25</td>
</tr>
<tr>
<td>4</td>
<td>28.07</td>
<td>26.78</td>
<td>12.21</td>
<td>46</td>
<td>-2.42</td>
<td>48.43</td>
</tr>
<tr>
<td>5</td>
<td>21.34</td>
<td>17.35</td>
<td>12.93</td>
<td>75</td>
<td>-9.58</td>
<td>40.92</td>
</tr>
<tr>
<td>6</td>
<td>27.93</td>
<td>34.38</td>
<td>7.56</td>
<td>22</td>
<td>17.55</td>
<td>52.76</td>
</tr>
<tr>
<td>7</td>
<td>94.70</td>
<td>88.55</td>
<td>12.04</td>
<td>14</td>
<td>64.26</td>
<td>115.10</td>
</tr>
<tr>
<td>8</td>
<td>46.85</td>
<td>49.56</td>
<td>16.85</td>
<td>34</td>
<td>13.83</td>
<td>88.69</td>
</tr>
<tr>
<td>9</td>
<td>12.62</td>
<td>15.89</td>
<td>4.08</td>
<td>26</td>
<td>8.82</td>
<td>27.45</td>
</tr>
<tr>
<td>10</td>
<td>22.18</td>
<td>26.32</td>
<td>5.90</td>
<td>22</td>
<td>14.34</td>
<td>39.48</td>
</tr>
<tr>
<td>11</td>
<td>83.78</td>
<td>82.03</td>
<td>15.09</td>
<td>18</td>
<td>35.43</td>
<td>111.20</td>
</tr>
<tr>
<td>12</td>
<td>38.96</td>
<td>38.81</td>
<td>6.59</td>
<td>17</td>
<td>22.41</td>
<td>49.21</td>
</tr>
<tr>
<td>mean</td>
<td>39.58</td>
<td>40.80</td>
<td>10.82</td>
<td>32</td>
<td>17.93</td>
<td>66.49</td>
</tr>
</tbody>
</table>

Two results of different calculations of plaque 8 are shown in figure 3.5. In these stress plots, there are both similarities and differences. The lipid core is visible on the right-hand side image. It is located on the left side of the plaque. The plot on the left-hand side shows very low stresses in the intima, except for two thin areas at the top and bottom of the lumen border. Furthermore, the stresses in the arterial wall are relatively high. The plot on the right-hand side shows an increase in stresses around the lipid core and at the top of the lumen border. Compared to the left plot, the stresses in the wall are lower. 95pCS of the left-hand and right-hand image are respectively 38.2 and 52.7 kPa.

Correlation of standard deviation of 95pCS and geometrical properties
For six geometrical properties, the correlation with the standard deviations in 95pCS was calculated. The geometrical properties are the minimum cap thickness, the maximum lipid core thickness, the lipid core angle, the mean lumen radius, the maximum lumen curvature and the maximum lipid core curvature. Two geometri-
Figure 3.6 shows the relation between cap thickness and standard deviation of 95pCS on the left-hand side. The Pearson correlation coefficient is -0.65. The right-hand side shows the relation between lumen radius and the standard deviation of 95pCS with a Pearson correlation coefficient of 0.65. Both correlations are statistically significant ($p < 0.05$).

Correlation 95p Cap Stress and cluster stiffness
To find the influence of the stiffness of different clusters on the 95pCS, Pearson correlation coefficients are determined for all plaques and clusters. 33 out of 48 are statistically significant ($p < 0.05$) and 13 coefficients have an absolute value of higher than 0.5. Table 3.2 on page 21 shows the results. The clustering for each plaque can be found in appendix A and the cluster sizes can be seen in table 3.4.

It would make sense to think that larger clusters have more influence on stress levels than small clusters. Therefore, the correlation between cluster size and cluster influence (as seen in table 3.2) was determined. Only one plaque showed a statistically significant influence of cluster size on cluster influence.

Two examples of influence of cluster stiffness on 95pCS are given in figure 3.7 on page 21. One example is given in which strong correlations exist between cluster stiffness and 95pCS and one example in which correlations are weak or absent. The results of the other plaques of this correlations analysis are also shown in appendix A.

Correlation 95p Cap Stress and region stiffness
To indicate the importance of stiffness per geometrical region, correlations between mean region stiffness and 95pCS are calculated. Table 3.3 on page 22 gives the Pearson correlation coefficients. The stiffness of the cap has a statistically significant effect on 95pCS in 11 out of 12 plaques. Of these 11 cases, 5 have a Pearson correlation coefficient higher than 0.5. The influence of stiffness of the shoulders and the back area on 95pCS are significant in 9 out of 12 plaques. For the remainder, 8 cases result in a significant influence. For the mean intima stiffness, 7 out of 12 cases are statistically significant.

For 6 out of 12 plaques, one or more correlations with absolute values higher than 0.5 are found. It is noteworthy that four of these plaques have 4 or 5 out of 5 correlation coefficients higher than 0.5.

Two examples are shown in figure 3.8 on page 22. Plaque 7 (top example) has high correlation coefficients for all geometrical regions, while plaque 9 (bottom example) only shows a weak correlation between cap stiffness and 95pCS.
Table 3.2: The Pearson correlation coefficients between cluster stiffness and 95pCS are determined for all 12 plaques for all 4 clusters. Only statically significant correlations (p<0.05) are shown. Absolute R-values higher than 0.5 are shown in bold font.

<table>
<thead>
<tr>
<th>Plaque nr.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>-0.29</td>
<td>-0.48</td>
<td><strong>-0.51</strong></td>
<td>0.23</td>
<td>-0.32</td>
<td>0.47</td>
<td>0.32</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.45</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>-</td>
<td>-</td>
<td><strong>0.65</strong></td>
<td><strong>0.76</strong></td>
<td>0.34</td>
<td><strong>0.64</strong></td>
<td><strong>0.79</strong></td>
<td>-</td>
<td>0.41</td>
<td>-</td>
<td>-</td>
<td>0.23</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>-</td>
<td><strong>0.96</strong></td>
<td>-</td>
<td><strong>0.81</strong></td>
<td><strong>0.88</strong></td>
<td>-0.23</td>
<td>0.30</td>
<td><strong>0.96</strong></td>
<td>-</td>
<td>0.45</td>
<td><strong>0.55</strong></td>
<td>0.44</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>-</td>
<td>-</td>
<td>0.27</td>
<td><strong>-0.61</strong></td>
<td>0.22</td>
<td>-0.33</td>
<td>-</td>
<td>-0.25</td>
<td>-</td>
<td>-</td>
<td><strong>0.74</strong></td>
<td><strong>0.56</strong></td>
</tr>
</tbody>
</table>

Figure 3.7: Two examples of the correlations between cluster stiffness and 95pCS are given. The top example is plaque 1. Only the red cluster has statistically significant influence on the 95pCS. For plaque 4, the results are given at the bottom. All clusters have a significant effect on the 95pCS. Three clusters (green, blue and yellow) have a Pearson correlation coefficient with an absolute value higher than 0.5. The two clusters that are dominant in the cap (blue and green) both have a strong positive correlation, while the yellow cluster has a strong negative correlation with 95pCS.
Table 3.3: The Pearson correlation coefficients between region stiffness and $95pCS$ are determined for all 12 plaques for all 4 regions. Also for the mean intima stiffness, correlations are determined. Only statically significant correlations ($p<0.05$) are shown. Absolute R-values higher than 0.5 are shown in bold font.

<table>
<thead>
<tr>
<th>Plaque nr.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap</td>
<td>-</td>
<td>0.26</td>
<td>0.28</td>
<td>-0.46</td>
<td>0.84</td>
<td>-0.30</td>
<td>0.89</td>
<td>0.94</td>
<td>0.37</td>
<td>0.37</td>
<td>0.69</td>
<td>0.72</td>
</tr>
<tr>
<td>Shoulders</td>
<td>-</td>
<td>-0.26</td>
<td>-0.36</td>
<td>-0.43</td>
<td>-0.31</td>
<td>0.73</td>
<td>0.80</td>
<td>- 0.41</td>
<td>0.65</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back</td>
<td>-0.27</td>
<td>-0.24</td>
<td>-0.39</td>
<td>0.73</td>
<td>0.80</td>
<td>- 0.40</td>
<td>0.77</td>
<td>0.26</td>
<td>-</td>
<td>0.28</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Remainder</td>
<td>-</td>
<td>-</td>
<td>0.30</td>
<td>0.25</td>
<td>0.30</td>
<td>- 0.40</td>
<td>0.76</td>
<td>0.71</td>
<td>-</td>
<td>0.35</td>
<td>0.67</td>
<td>0.92</td>
</tr>
<tr>
<td>Intima</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.24</td>
<td>-0.24</td>
<td>0.83</td>
<td>0.73</td>
<td>-</td>
<td>0.37</td>
<td>0.65</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Figure 3.8: Two examples of the correlations between geometrical region stiffness and $95pCS$ are given. The cap is shown in red (top-left plot), the shoulders in green (top-right plot), the back in blue (bottom-left plot) and the remainder in yellow (bottom-right plot). The upper example is plaque 7. All regions show a strong correlation with $95pCS$. For plaque 9, the results are given at the bottom. Only the cap stiffness is correlated to the $95pCS$ for this plaque.
Table 3.4: The clustering that was performed for all 12 plaques led to different cluster sizes. In this table, the relative cluster sizes of all clusters of all plaques are given.

<table>
<thead>
<tr>
<th>Plaque</th>
<th>Red Cluster Size</th>
<th>Blue Cluster Size</th>
<th>Green Cluster Size</th>
<th>Yellow Cluster Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.4%</td>
<td>31.2%</td>
<td>16.3%</td>
<td>22.1%</td>
</tr>
<tr>
<td>2</td>
<td>28.9%</td>
<td>50.7%</td>
<td>20.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>3</td>
<td>36.7%</td>
<td>19.3%</td>
<td>12.7%</td>
<td>31.3%</td>
</tr>
<tr>
<td>4</td>
<td>12.0%</td>
<td>5.2%</td>
<td>33.4%</td>
<td>49.5%</td>
</tr>
<tr>
<td>5</td>
<td>39.0%</td>
<td>2.7%</td>
<td>23.5%</td>
<td>34.9%</td>
</tr>
<tr>
<td>6</td>
<td>7.6%</td>
<td>10.9%</td>
<td>42.3%</td>
<td>39.1%</td>
</tr>
<tr>
<td>7</td>
<td>26.4%</td>
<td>39.5%</td>
<td>31.4%</td>
<td>2.7%</td>
</tr>
<tr>
<td>8</td>
<td>15.1%</td>
<td>8.6%</td>
<td>50.4%</td>
<td>26.0%</td>
</tr>
<tr>
<td>9</td>
<td>1.7%</td>
<td>27.0%</td>
<td>29.0%</td>
<td>42.3%</td>
</tr>
<tr>
<td>10</td>
<td>8.2%</td>
<td>4.9%</td>
<td>50.7%</td>
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</tr>
<tr>
<td>11</td>
<td>16.0%</td>
<td>11.4%</td>
<td>58.6%</td>
<td>14.0%</td>
</tr>
<tr>
<td>12</td>
<td>23.3%</td>
<td>3.5%</td>
<td>34.6%</td>
<td>38.6%</td>
</tr>
</tbody>
</table>

**Effect of different cluster distribution**

For plaque 11, the effect of a different clustering and different stiffness set is tested. This new cluster is not based on the k-means clustering algorithm. All grayscale values of the intima are divided in four equally sized clusters. After assigning pixels to clusters, the same erosion and reconstruction steps are made to remove isolated pixels. Due to these steps the final clusters are no longer equal in size. Since the original clustering method with the k-means clustering algorithm and the new clustering are both based on grayscale-values, the two clusterings show strong similarities.

Figure 3.9 gives a comparison between the original and the new clustering and gives the range of 95pCS for both clusterings. The mean (± std) stiffness of the original and new clustering are respectively 82.0 (± 15.1) and 78.6 (± 23.0) kPa.

![Figure 3.9](image-url)
Single cluster variation
To further investigate the influence of stiffness of a single cluster on the 95pCS, single cluster variations for two plaques were performed. Three clusters were given the mean intima stiffness of 448.5 kPa and the fourth cluster stiffness varied from 6 up to 891 kPa. With this method, the influence of the other clusters is eliminated. The yellow clusters of plaque 3 and plaque 10 were chosen for this analysis, because these clusters are present around a large part of the lumen border. In table 3.2 it can be seen that the previously found correlation coefficients of plaque 3 and 10 were respectively 0.27 and not significant. It was hypothesized that eliminating influence of the other clusters would lead to a strong positive correlation between the yellow cluster stiffness and 95pCS.

Figure 3.10 shows the results of the analysis. For plaque 10 (right-hand side image) two extra calculations were performed with higher stiffnesses, because a local minimum seemed to be present at around 750 kPa. For plaque 3, the correlation is 0.96. For this plaque, the hypothesis is confirmed. For plaque 10, the hypothesis is rejected. Based on the first 10 simulations (with stiffnesses in the original range of 6 to 891 kPa) the correlation coefficient is -0.91. Adding the two extra calculations led to a non statistically significant correlation coefficient.

Figure 3.10: On the left-hand side the single cluster analysis of the yellow cluster of plaque 3 is shown. The right-hand side shows the same for plaque 10.
4. Discussion & conclusions

Rupture of atherosclerotic plaques is the main cause of ischemic events, such as strokes and heart attacks. It is therefore crucial to know which plaques have a high rupture risk and which don’t. Currently used diagnostics have a limited value in predicting the rupture risk and therefore new methods are being developed. A promising approach is to estimate the stress levels in the plaque with the use of biomechanical modeling. Current research usually performs FEA with homogeneous sections, such as the intima, lipid core and arterial wall. Especially the atherosclerotic intima is known to be very heterogeneous of nature. Therefore, two goals were set for this study:

The first goal is to create a method that allows to model heterogeneity in the intima.
The second goal is to find the influence of intima heterogeneity on stresses in the artery.

Both goals were met in this study and will be discussed in this chapter. The new approach in which heterogeneity is added will be discussed in section 4.1 and the influence of heterogeneity on stress levels will be discussed in 4.2.

4.1 Creating heterogeneous FEA models of atherosclerotic arteries

The heterogeneous approach that was created in this study consists of two main parts. The creation of a stiffness map of the artery and the implementation of this stiffness map in FEA software. In this study, the stiffness map is created in MATLAB and the FEA software is ABAQUS. Heterogeneity was only applied in the intima, since this is the most heterogeneous part of the atherosclerotic artery. However, the newly created method can also be applied to introduce heterogeneity in any other part of the artery.

With the new approach, a mesh was created that is independent of the geometry within the artery. The validity is discussed in section 4.1.1. A discussion on the addition of heterogeneity to biomechanical modeling of plaques is given in 4.1.2.

4.1.1 Validity of segmentation independent approach

The new methodology in using FEA on atherosclerotic arteries uses a mesh that is independent of the manual segmentation of the different components in the mesh. Furthermore, smoothing is applied on the material stiffness to reduce numerical errors. Figure 3.2 showed a comparison of the stress results of homogeneous calculations of the conventional and the new approach.

Generally, the resulting stiffness maps of the conventional and the new approach are very similar. The representative example of figure 3.2 shows equal locations of local peak stresses in both the wall and in the intima. The magnitudes of these local maxima show minimal variations.

However, due to the smoothing that is applied on the material properties in the new approach, local maxima at the border of the lipid core are of a lower magnitude or even disappear in the new approach. Although it is the current practice to use the conventional approach, there is no evidence that the discontinuities in material stiffness of the conventional approach is more realistic than the smoothed material properties. The smoothing potentially gives a more realistic material stiffness map. Since the validation of the new methodology showed only minimal differences between the conventional and the new approach, the new approach is seen as valid to investigate the influence of heterogeneity.

The influence of smoothing on stress levels was not investigated in detail in this study. More numerical studies on the effect of smoothing and more experimental studies on local material properties will lead to a better understanding and potentially answer the question on whether or not to perform smoothing on material properties.
4.1.2 Generating heterogeneous FE models

Based on the new approach, material properties were assigned to individual elements in the FEA model. This gives the potential to create a fully heterogeneous atherosclerotic plaque model. In this study, only heterogeneity was allowed in the intima, since this is known to be the most heterogeneous part of the artery [8]. This is the first study to actually model heterogeneity within FEA on plaques.

Based on the histology images that were available for this study (see appendix A), even for an experienced pathologist it would be very hard to perform a manual delineation of different components in the plaque. Therefore, an automated clustering method was written specifically for this study. Based on color intensity of the histology images, four clusters were defined within the intima. Color intensity does not have a relation with material stiffness. Therefore, the clustering does not give a one-on-one translation from actual stiffness to model stiffness, but it does give a realistic cluster distribution.

To be able to create stiffness maps that give better one-on-one translations of actual stiffness, future studies on heterogeneity should optimize their image acquisition, so that different components can be distinguished. One way to achieve this, is to perform different staining techniques on a single plaque. If the different staining techniques each highlight different components in a plaque, such as calcifications and collagen, a manual delineation can be performed. This will lead to a better stiffness representation.

For each of the 12 plaques in this study, 100 sets of stiffnesses were defined with Young’s moduli ranging from 6 up to 891 kPa, as reported by Chai et al. [9]. The 100 sets were created with the use of a Latin Hypercube. In these sets, worst case scenarios are also included in which part of the intima is very rigid, while other parts are very soft. To remove these scenarios, 10 simulations with the highest change in lumen area and 10 simulations with the lowest change in lumen area were discarded. This led to a removal of the most extreme and unrealistic cases.

4.2 The influence of heterogeneity on 95pCS

High stresses in the cap of an atherosclerotic artery can lead to the rupture of a plaque. Biomechanical modeling of these peak cap stresses gives an estimation of the rupture risk of the plaque. Current studies on calculating these peak cap stresses all use homogeneous models. It is a logical assumption, but it is unknown how accurate the peak cap stresses are calculated with this assumption. This study compared homogeneous calculations with heterogeneous calculations to study the influence of heterogeneity on peak cap stresses.

Homogeneous vs Heterogeneous approach

The most important finding of this study is that adding heterogeneity to plaque stress calculations has a considerable effect on 95pCS. As can be seen in the boxplots of figure 3.4, for each of the twelve evaluated plaques, the 95pCS varied considerably throughout the different heterogeneous calculations. Some plaques showed more variation than others. Standard deviations relative to their mean, ranged from 14% to 75%. The high levels of variation clearly indicate that the homogeneous assumption can not be made without losing accuracy in calculating peak cap stresses to determine rupture risk.

For most of the 12 plaques, the mean 95pCS of the heterogeneous calculations was close to the homogeneous 95pCS, the mean difference is $5 \pm 15.8\%$. This indicates that the homogeneous calculation does give an estimate in the same order of magnitude as the heterogeneous calculations.

Determining the variation induced by heterogeneity by running 100 simulations for a single plaque (as was done in this study) is very time consuming and is therefore not optimal for a clinical setting. It would be more valuable to be able to predict the variation, rather than having to calculate it. A logical first step is to find a relation between the magnitude of the homogeneous calculation and the variation for that plaque that was found with the heterogeneous calculations. In this study, no relation between the two was found. Therefore, the influence of other factors on variation were investigated.

Correlation of standard deviation of 95pCS and geometrical properties

A second step to determine the uncertainty range of 95pCS for a plaque, was to relate geometrical properties of a plaque to the variation in 95pCS that was found. The geometrical properties that were evaluated are the minimum cap thickness, maximum lipid core thickness, lipid core angle, lumen radius, maximum lumen curvature and maximum lipid core curvature. These properties can be deduced directly from imaging modalities, such as MRI or ultrasound. From these six geometrical properties, two showed a significant
Correlation with variation in 95pCS: minimum cap thickness and lumen radius. A thinner cap and a larger lumen radius leads to more variation in 95pCS.

Cap thickness showed a negative Pearson correlation coefficient of -0.65. This indicates that a thinner cap leads to more variation in 95pCS for the heterogeneous calculations. To be able to explain this phenomenon, think of a simplified idea in which stresses $\sigma$ are described by stiffness $E$ and deformation $\delta$, see equations 4.1.

$$\sigma = E\delta$$  \hspace{1cm} (4.1)

Consider a plaque with a very thin cap; the deformation ($\delta$) of this plaque would not be greatly influenced by the stiffness ($E$) of this cap, since it is thin and therefore has little constructional effect. In an extreme situation: regardless of the stiffness of a thin cap, the deformation is the same. So a high stiffness leads to a high stress and a low stiffness leads to low stress. If you would consider a thicker cap, the stiffness of this cap would have a larger influence on the deformation. A stiffer cap would lead to less deformation and a softer cap would lead to more deformation. At this situation, stiffness and deformation are negatively related to each other and therefore the stresses of stiff and soft thick caps will show less difference.

Lumen radius showed a positive Pearson correlation coefficient of 0.65. In other words, a larger lumen leads to more variation of heterogeneous 95pCS. Consider two plaques with equal outer dimensions, but one with a small lumen and one with a larger lumen. The plaque with a larger lumen has a thinner artery than the plaque with a small lumen. Potentially, the same reasoning as above for thin caps holds true for thinner arteries, therefore explaining why a larger radius leads to more variation.

Although two relations between geometry and variation in 95pCS were found, these two factors alone do not predict the variation very well. Also the reasoning for the correlation is speculative. To further be able to predict the variation of 95pCS, more studies with more plaques should be included. Another limitation of the current study is that a univariate analysis was performed. Extending this to a multivariate analysis might help in predicting the variation of 95pCS, based on geometrical properties.

Correlation 95p Cap Stress and cluster stiffness
In this study, the intima was divided into four different clusters. The relations between clusters and 95pCS was statistically investigated. The correlations between cluster stiffness and 95pCS were intended to develop a sense of mechanical importance per cluster.

Since the relation between peak cap stress and cluster stiffness is determined, it makes sense that stiffness of clusters that are present in the cap would have a positive correlation with the 95pCS. In most cases, this is indeed the case. Especially in plaques with thinner caps, the stiffness of the clusters that are dominant in the cap have a relatively strong correlation with the 95pCS. Although this was found to be true for many cases, it was not always true. Some clusters that are mainly in the cap did not show a correlation with the 95pCS and some clusters that are not present in the cap at all did show a positive correlation.

Another hypothesis was that stiffness of clusters that are dominant in the area behind the lipid core would have a negative influence on 95pCS. If this area behind the lipid core is soft, it would allow for more deformation of the lipid core and the cap. Only two relatively strong negative correlations were found and both of these were of clusters that were indeed dominant in the area behind the lipid core.

More hypotheses were formed and tested in this analysis. It was for example thought that shoulder stiffness would influence the deformation pattern, hence the stresses, in the cap. Yet another idea was that if a cluster would be present around the entire lumen border, it would have strong influence on the deformation and the stresses in the plaque. These hypotheses could not be confirmed and sometimes the influence was exactly opposite of what was expected to be. This indicates that the calculations are too complex for mechanical intuition to be able to predict the influence of a single cluster.

Investigating the relations between cluster stiffness and 95pCS has led to the two ideas that cap stiffness is positively correlated with 95pCS and that the stiffness of the area behind the lipid core is negatively correlated to 95pCS. However, due to the irregular shapes of the clusters it is hard to objectify these ideas.

Correlation 95p Cap Stress and region stiffness
Since it proved to be difficult to determine the influence of clusters based on their location, the average stiffness per geometrical region was determined. The correlations between these average stiffnesses and 95pCS were also determined. The four geometric regions in this study are: the cap, the shoulders, the back (area behind the lipid core) and the remainder (see figure 2.9). It is valuable to know which of these regions have the
most influence on the 95pCS. Future studies on patient-specific stress analysis can then focus on finding the stiffness of these regions to increase the accuracy of their simulations.

Due to the setup of this study, the stiffness of the four intima regions were dependent on the same set of clusters. Therefore, the stiffness of the geometric regions were correlated to each other. This resulted in the fact that either (mostly) all or (mostly) none of the regions had a significant correlation with 95pCS. To identify the influence of region stiffness, future studies should vary stiffness per region and not per cluster, even though this is not a good representation of the reality.

Even though the setup was not optimized for finding the influence of region stiffness on 95pCS, it was found that the stiffness in the cap generally had the largest influence on the 95pCS, just as was expected and found in the previous analysis in which cluster stiffness and 95pCS were analyzed.

**Effect of different cluster distribution**

One of the key elements in this study is the choice for clusters. Different cluster distributions could very well lead to different findings in terms of 95pCS. Therefore for one plaque a different clustering was created and the stress results were compared to the original clustering.

The new clustering was based on grayscale values, just as the original clustering. This led to a clustering that was certainly different, but did show similarities as well. The different clustering led to minimal changes in 95pCSs, indicating that the range that was found in this study is not strongly dependent on cluster distribution.

To further investigate the influence of the chosen cluster distribution, a future study should compare multiple different clusterings for each plaque. The clusterings should not all be based on grayscale values so that the clusters are less similar to each other.

**Single cluster variation**

One of the hypotheses that was mentioned earlier, was that if a cluster would be present along the entire lumen border it would affect the deformation of and therefore the stresses in the plaque. The idea behind this hypothesis is that for similar deformations, the stresses would be high in this ‘ring’ if the stiffness is high and the stresses would be low for a low stiffness.

Two clusters of different plaques that are both present around a large part of the lumen border were chosen for a single cluster variation analysis. The stiffness of the other clusters in the intima were set at a constant to cancel out their effect on the 95pCS and the variation in results was purely caused by the single chosen cluster.

For one of the two clusters the hypothesis can be confirmed, while for the other the hypothesis can be rejected. This analysis indicates again that stress calculations on plaques are too complicated for mechanical intuition and underlines the necessity of performing FEA to estimate stresses in atherosclerotic arteries.

### 4.3 Conclusions and recommendations

Rupturing of atherosclerotic plaques can lead to serious health issues. It is therefore crucial to be able to predict the rupture risk. Since current clinical diagnostics are of limited value in determining the risk, biomechanical modeling is being developed as a better tool in determining the rupture risk.

It is well known that biological tissue is far from homogeneous. Especially the intima of atherosclerotic arteries shows a high level of heterogeneity. Modeling this heterogeneity is not an easy task for multiple reasons. Finite Element software uses homogeneous sections, so a workaround is needed to be able to introduce heterogeneity. Besides the computational challenges, the actual heterogeneity of a plaque needs to be known if you want to model a specific plaque. There are no current methods that are able to accurately capture the heterogeneity of a single plaque.

In this study a first step is set in the direction of adding heterogeneity in biomechanical modeling of atherosclerotic arteries. A new approach for a segmentation-independent FE model was created in which each element can have its own stiffness parameters assigned to it. Any heterogeneous distribution can therefore be modeled with this approach.

Besides creating a new method that includes heterogeneity in stress calculations, this study also investigated the influence of heterogeneous material properties. It was found that heterogeneity has a strong influence on the stress levels, especially in the cap. Omitting heterogeneity in determining the rupture risk of a plaque therefore results in less accurate results.
This study based the heterogeneous material distribution on image intensity levels of histology images of 12 different plaques. This method does not provide one-on-one translations of the real plaque behavior. Since it is shown that heterogeneity has a strong influence on stress levels, it is recommended to develop a way of capturing actual heterogeneous material behavior of plaques. The first steps into this direction can be made by performing more µ-indentation tests on ex vivo human arteries. If the stiffness distribution is accurately captured, stress calculations of this plaque can be done with both homogeneous and heterogeneous distributions to find the actual difference in peak cap stress.

The approach that was taken in this study was a 2D approach. Since arteries are 3D structures, the new approach can be expanded by future studies by adding the third dimension to it. Apart from strongly increased calculation times, no problems in adding a this third dimension are currently expected.

In conclusion, a new way of performing Finite Element Analysis on atherosclerotic arteries was created in this study. Heterogeneity was successfully added to the intima and this heterogeneity was found to have a strong influence on peak cap stresses. This study opens up the field of heterogeneity in biomechanical modeling of atherosclerotic arteries and thereby adds value to creating an improved diagnostic tool to determine rupture risk on atherosclerotic arteries.
Bibliography


Appendix A: All histology images and cluster distributions

In the following pages of this appendix, all histology images of the 12 plaques are shown. Also the clusters of the intima are shown and the correlations between cluster stiffness and 95pCS is shown. The clustering and correlation images were an inspiration in finding relations between important locations and 95pCS.
Figure A.1: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 1.
Figure A.2: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 2.
Figure A.3: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 3.
Figure A.4: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 4.
Figure A.5: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 5.
Plaque 6

Figure A.6: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 6.
Plaque 7

Figure A.7: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 7.
Figure A.8: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 8.
Figure A.9: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 9.
Figure A.10: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 10.
Figure A.11: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 11.
Figure A.12: Histology, clustering and cluster stiffness correlation with 95pCS of plaque 12.