KING'S COLLEGE LONDON
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Third Year Project in Physics

Bending or Indenting?

Modelling the nanoindentation testing of collagen to explore the nature of experimental deformations

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I declare that this report is my own work and is not plagiarised.
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Abstract

AFM nanoindentation is an experimental technique that can test the mechanical properties of materials at the nanoscale. In this case, the method investigates the suitability of the protein collagen for possible applications in tissue engineering. This report details the creation of a computational model in Ansys to recreate and explore the nature of results from a lab-based experiment. In particular, how both the bending and the indentation of the collagen fibril during testing contribute to the experimentally-obtained indenter tip displacement. A basic geometry for the model was developed, and a finite element analysis was conducted to simulate the physics of the scenario. Care was taken to implement appropriate modelling parameters such as boundary conditions and symmetry planes.

A successful simulation was created, which showed both bending and indenting of the fibril. This implies that the experimental tip displacement cannot be assumed as purely the indentation or bending depth in any calculations. The ratio of bending to indenting was found to be consistently around 90% to 10%. The accuracy of the simulation compared to the experiment is considered, in addition to the impact of optimising the modelling parameters. The implications of these results will be discussed, as well as potential improvements and avenues for future investigation.

Contents

1 Introduction

A bridge between the fields of engineering and biology has been growing rapidly. Traditional engineering methods are increasingly being utilised for medical applications. An upcoming area within bioengineering is tissue engineering - a field that applies concepts from engineering and the life sciences "toward the development of biological substitutes that restore, maintain or improve tissue function or a whole organ"[1].

The value of this field of research is apparent when considering the statistics on organ donation. For example, in the UK in 2020 there were over 6000 people on the waiting list for an organ transplant. Of those, 377 died before they received a transplant [2]. Despite the scarcity of donor organs, the only long-term option for many types of organ failure is to wait for a transplant. Additionally, transplantation hosts its own issues, as the battle to prevent the immune system from rejecting a donor organ is lifelong [3]. The loss or failure of organs or tissues is one of the most frequent, devastating and costly problems in human healthcare, so the ability to create tissues artificially would be revolutionary [1].

Many different approaches are taken to develop artificial organs - often depending on the requirements for the specific organ needing replacement. Synthetic materials have been used, often to varying degrees of success. However, the need for biocompatibility - the ability to exist in conjunction with the body without causing harm - means the use of biological materials will always take precedence [4]. Tissue engineering allows functional living tissue to be created using living cells (i.e. stem cells), usually associated with scaffolding to guide tissue development [3].

These so-called "scaffolds" play a massive role in the success of tissue engineering. They mimic the function of the extracellular matrix (ECM) in the body. The ECM provides an essential physical structure for the cells of a tissue and initiates crucial biochemical and biomechanical cues for a range of processes [5]. It is fundamental in any tissue engineering endeavour to find an appropriate material to create a functional scaffold.

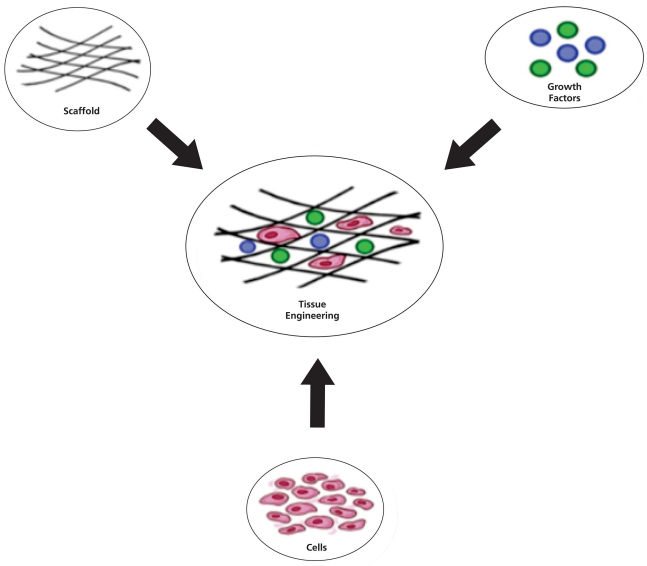

Figure 1 *A diagram showing the basic components of a tissue engineering construct [10].*

The collagens are a family of ECM macromolecules that are found everywhere within the body. They contribute to many tissues' mechanical properties and biological function, ranging from bone to the cornea. While there are many types of collagen, Types I, II and III are the most common within the body. Type I is the most abundant macromolecule within the ECM [8]. Collagens act as supporting scaffolds to maintain tissue structure and anchoring devices to promote cellular adhesion to non-cellular areas [8]. Collagen naturally performs many of the functions desired in a scaffolding material, making it an ideal candidate.

While the variety and complexity of biological materials such as collagen provide significant benefits in tissue engineering, there are also difficulties to consider. As briefly mentioned, the scaffold can be used to shape and influence the resulting tissue. This is a complex process within the body, so fitting the specific requirements for the required tissue is vital in any scaffolding material. Therefore it is essential to have accurate results for the material properties of the collagen used - most notably the mechanical properties. Therefore, a reliable source of data on the mechanical properties is vital for scaffold construction; however, this is not straightforward to obtain.

This is where biology can look to engineering for inspiration. Structural analysis is a field of engineering used to test the effects of loads on structures

and their components. Mathematical models are used to calculate material properties such as stress, strain and deformations - ideal properties to determine for the scaffold. Indentation hardness testing is an example of a structural analysis method that can ascertain the 'hardness' of a material - its ability to resist permanent deformation [11]. This is a well-established technique with many variants and applications. In general, the process uses an indenter that is pressed into a material by a load, forming a permanent impression on the sample. The hardness is then given by the load divided by the area of the indentation [11].

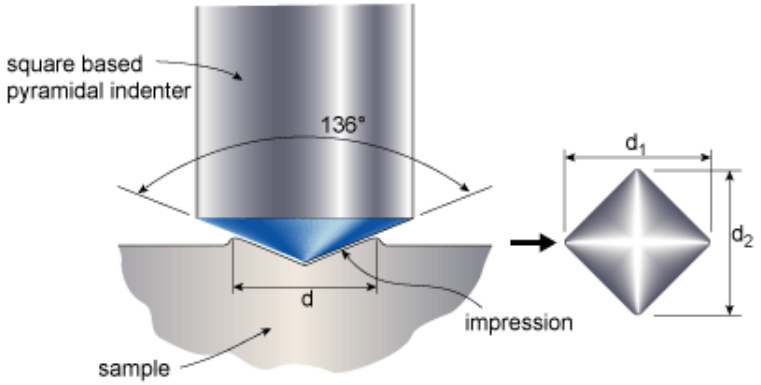

Figure 2 *A diagram showing a variant of indentation hardness testing, indicating how the indentation impression can be measured [12].*

Traditionally, structural analysis ensures the stability of large structures such as buildings, bridges or planes. However, the method can also be applied to much smaller materials - including collagen - but adjustments must be made.

A relevant adaptation of indentation testing is nanoindentation - a method closely linked to its more established counterpart. It solves a key issue preventing testing the mechanical properties of collagen with the traditional process outlined above. Namely, that collagen is too small for that type of testing. The average type I collagen fibril has a diameter on the order of tens of nanometers [13]. An important difference in nanoindentation is that the impression cannot be directly measured due to the nanometer scale. SEM or similar methods could be used to image this area. Still, most often, the area of contact is determined by measuring the depth of penetration of the indenter into the sample. Together with the known geometry of the indenter

tip, a value for the impression area and then the hardness can be calculated [14].

While this is a considerable improvement on other methods, it does not come without flaws. The most significant is that the testing is too small to see. While indirect methods of calculating the impression area are helpful, they do not provide information on how the testing looks. If the investigator cannot see how the materials deform throughout testing, it can be more challenging to interpret the results. This project aims to find a solution that will allow scientists to analyse nanoindentation testing visually. This will be achieved by creating a computational simulation of a nanoindentation experiment on collagen that was carried out in a lab. This experiment used an atomic force microscope (AFM) with an indentation tip to indent the collagen across various forces. For each force, a maximum tip displacement was measured.

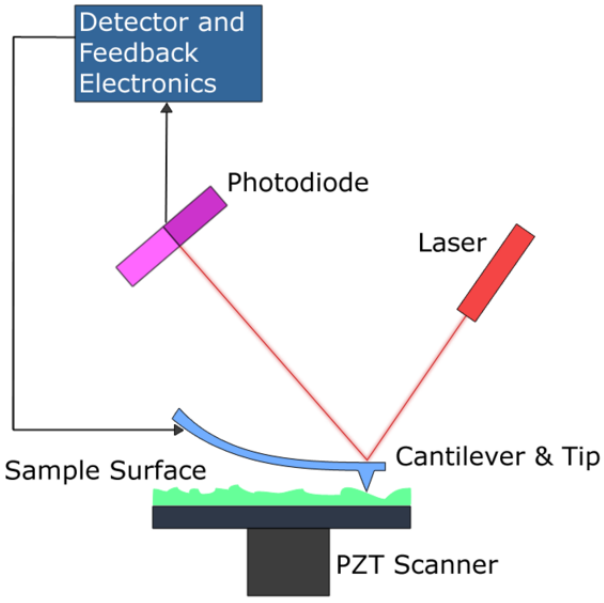

Figure 3 *A diagram showing the major components of an AFM setup [16].*

AFM is a type of microscope that images samples by 'feeling' with a cantilever tip. The tips' mechanical motion is translated into usable data using a laser and photodiode system [15]. AFM has many applications, but in this case, a 'force mode' is utilised. This brings the AFM tip into contact

with the sample surface, pushed to a maximum load and then withdrawn [18]. In the lab, a collagen fibril was prepared for the testing on a surface of the polymer polydimethylsiloxane (PDMS) - a widely used silicon-based organic polymer [17].

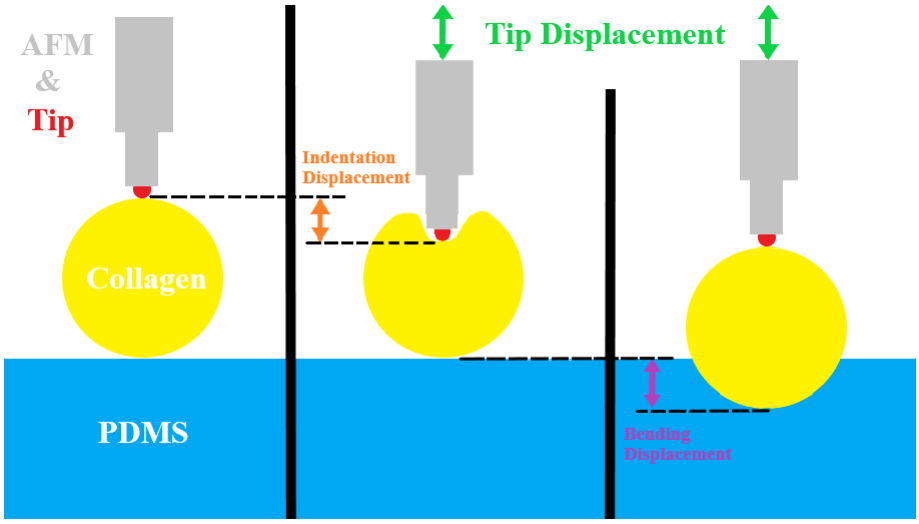

Figure 4 *A self-drawn diagram illustrating the fundamental measurements of the investigation (not to scale).*

Creating a computer simulation of this experiment means the indentation process can be seen and quantified no matter the scale. Specifically, this investigation will use the simulation to consider the relationship between the deformation of the collagen fibril with the material is placed on - in this case, PDMS. As mentioned, nanoindentation can calculate mechanical properties such as hardness using the indentation depth at a given load. However, this assumes that the indenter tip displacement is the same as the indentation depth into the fibril. Without visual confirmation, it is unclear whether the PDMS beneath the collagen is also deforming and how this affects the measured tip displacement. It is hoped that the simulation could provide information shown in Figure 4. The middle panel shows a visual and quantative measurement for the indentation displacement and the right panel shows the sample for a bending displacement.

A finite element analysis (FEA) method shall be used, as is common in structural analysis simulations. A finite element method (FEM) is a numerical computational method for solving differential equations - in particular,

the PDE's that describe the structural behaviour of a scenario. By discretising the model into finite elements (a.k.a. a mesh), these infinite-dimensional equations are transformed into a set of linear equations that can be solved by a computer [11]. The main interaction of this investigation with FEA comes from refining the finite element mesh used in the simulation setup.

The goal of this investigation is to recreate the experimental results as closely as possible, as well as determining a ratio between the bending and indenting of the fibril.

2 Method

The starting point for any computational modelling exercise is to consider how best to recreate the scenario in question. In our case, experimental results were provided by PhD student Emilie Gachon, who carried out some nanoindentation testing of collagen in a lab. A summary of this information is provided in Appendix A to prevent repetition. This data was used to set up and test the model. To determine the amount of bending or indenting, we are interested in the mechanical properties of the experiment - in particular, the stress, strain and deformation.

The popular simulation software Ansys was chosen, which allows for mechanical analysis. The aim of using this software was to provide accurate data and a visual representation of how the objects deform. Our team was divided into two by software choice between Ansys and a similar cloud-based version, Simscale. Ansys proved more useful than Simscale, so details of Simscale will not be included further in this report for brevity. Details of those results can be found in my colleagues' reports.

2.1 Ansys Model Setup

Here, an overview of the basic setup process for this model in Ansys is given. The goal here was to create a viable recreation of the experiment that would reproduce those results as closely as possible. The discussion here details the options chosen during the setup process and any modelling assumptions made, but the final parameters chosen can be found in Section 2.4.

2.1.1 Analysis Method

Initially, an analysis system must be chosen in the Ansys 'Workbench' programme. In order to carry out a FEA, the Static Structural analysis option was chosen. This creates a structural analysis that does not change over time. This investigation focused on reproducing the experiment at maximum loading rather than the loading/unloading process so this option was most appropriate.

2.1.2 Material Properties

The material parameters are defined in the 'Engineering Data' section. It is possible to either use pre-defined materials or create new materials by

defining their properties. Using as many values from the experimental data as possible improves the accuracy of the model. Therefore, three different materials were defined - for the indentation tip, collagen fibril and PDMS substrate. Not all properties of the material need to be defined - only those relevant to the scenario. In this case, properties were described using the Isotropic Elasticity format. This required values for the Young's modulus and Poisson ratio of each material. The Young's modulus of a material is the ratio of stress to strain. In contrast, the Poisson ratio quantifies the deformation of a material in directions perpendicular to the specific direction of loading [11].

Defining constant values using the 'isotropic elasticity' tool makes two assumptions. The first is that the materials have the same properties in all directions - so are isotropic. The second is that the material deforms linearly, meaning the relationship between stress and strain is constant. The simplification of the geometry described later allows for an assumption of isotropy in the materials. In the case of linearity, this experiment involves deformations that are small compared to the body in question. This makes linearity a reasonable modelling assumption.

2.1.3 Geometry

The next step was designing an appropriate geometrical representation of the scenario. This was created on SpaceClaim CAD software using nanometer units. Geometries can be made by creating a 2D sketch on an appropriate plane and extruding to 3D shapes. These shapes can then be aligned for correct placement. The main factor here is to consider what features are necessary to reflect the critical parts of the experiment. Simplifying the geometry makes the modelling process less complex and reduces the computational power needed to run the simulation.

The experimental setup was separated into three components - a fibril of collagen, the PDMS surface it rests on and the indenter tip. Since a force can be applied to the top of this tip, modelling any other aspects of the AFM setup would be redundant. The PDMS was modelled as a basic cuboid - since any variation in its shape would be beyond the scale of this testing. The indenter tip was modelled as a semi-sphere. This allowed a force to be applied evenly across its flat surface. While the exact shape of the indenter tip can vary, a rounded tip is more appropriate for biological materials [9].

Collagen has a complex structure, but it is not necessarily relevant for this

investigation. Due to the time limitations on this project, it made the most sense to test a basic cylinder geometry - since a fibril diameter was provided with the experimental data. More complex shapes would require more FEA elements to be used, which would be more useful elsewhere. Additionally, an appropriate choice of Young's modulus could average could the variations in material properties along the length of the fibril. As was shown by a colleague Xiaolin, recreating complex geometry does not always give more accurate results. More details on this can be found in his paper.

The other consideration when setting up the geometry was how much needed to be simulated. It is common practice in computational modelling to half or quarter the geometry along planes of symmetry. Since the geometry deforms in the same manner on either side of these planes, the computational power required can be massively reduced by only modelling a relevant section of the geometry.

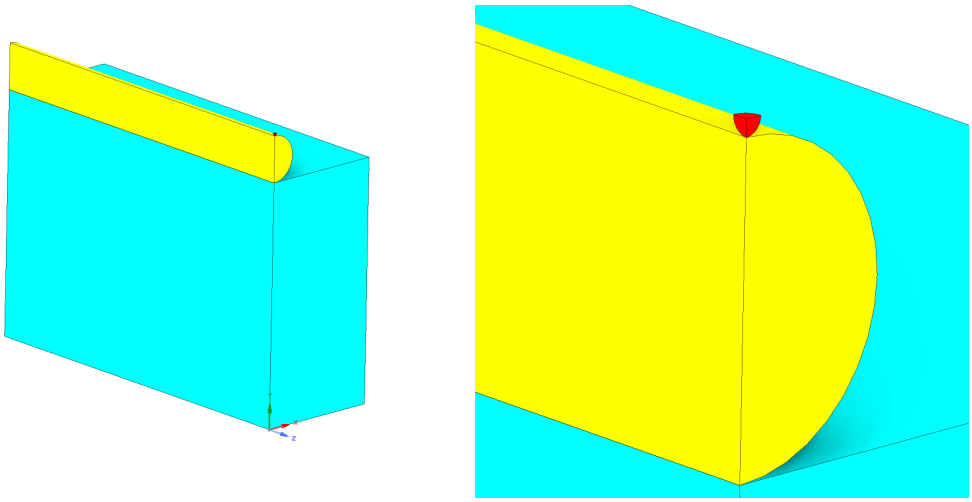

Figure 5 *Screenshots from SpaceClaim showing the quartered geometry used in this investigation - with the blue PDMS, yellow collagen fibril and red indender tip.*

A quartered geometry, as shown above, was chosen for this simulation to maximise FEA elements for the most important areas. Appropriate boundary conditions must be used to give equivalent results to a full simulation. In this investigation, the 'Symmetry' function was applied to all three objects along the planes of symmetry shown above - namely the x and z planes.

These boundary conditions restrict any movement of the object normal to the direction of the force applied. This symmetry feature could also be used in future investigations via the linear periodic setting for more complex collagen geometries such as OG regions. A key consideration when quartering the geometry is how this affects the physics of the scenario. In this case, the forces applied on the tip must be quartered since force is a function of pressure and area.

2.1.4 Modelling Parameters

Finally, the modelling parameters can be set up. The created materials are assigned to the appropriate geometries. The other boundary conditions to specify are the supports. These are the surfaces that restrict the movement of the whole system in space. Fixed supports were applied along all outer planes besides those assigned to the symmetry. Contacts define the relationship between components, i.e. the tip, fibril and surface. There are many types of contact in Ansys, but only the *bonded* and *no separation* contacts apply to a linear analysis. The difference between these two contacts is that the latter allows sliding between parts. Some testing showed a minimal difference between the results using each type of contact. Although it may require more computational power, this investigation chose no separation contacts as they better reflect reality.

An essential part of this model is the meshing, which plays a crucial role in FEA and can significantly influence results. This will be investigated in a later section, but an option to automatically generate a mesh is available in Ansys. A force parameter can be added, acting downwards on the top of the tip. Its magnitude and direction can be controlled.

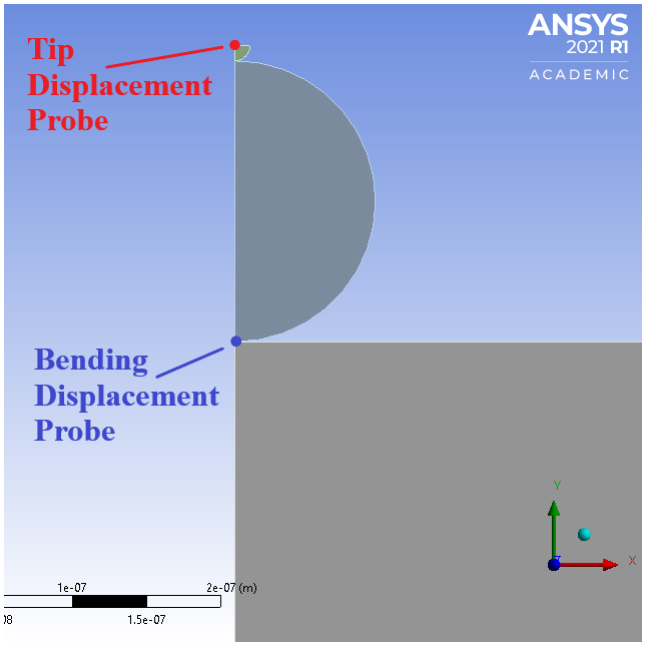

Figure 6 *A labelled screenshot indicating the locations of the chosen measurement probes.*

The only remaining step is to define the desired outputs from the simulation. The main aim of this project is to quantify how much each component deforms. Therefore the chosen method of taking measurements must be carefully considered. It must be clear what the measurement represents while being as accurate as possible. In addition to general deformation measurements and visualisations, Ansys allows the placement of specific deformation probes for a point, plane or even body. This can quantify either the total or directional displacement.

As shown in the figure above, two point displacement probes were chosen to measure the tip displacement and the displacement of the PDMS. It was found that the bodies themselves may deform during the simulation - including the tip. Therefore, it would not be accurate to measure the average displacement across a body if its shape and size may change. In the case of the tip, it was important for this point to be placed on top of the tip rather than below for the same reasons.

2.2 Finding Optimal Parameters

As in any computational modelling endeavour, there is a trade-off between accuracy and efficiency. A detailed model covering all complexities in the geometry and physics of the scenario would be ideal; however, computational power is not unlimited. Limiting factors such as the processing power or the number of FEA elements must be considered in simulation design. It is not always necessary to have a perfect recreation of a scenario to obtain the results desired. Testing can be carried out to determine at what point improving the accuracy of a particular simulation parameter stops improving the usefulness of the results. For example, the experimental data recorded a PDMS depth of 1mm and a maximum tip displacement of 1116nm. With a difference in scale of this size, it seems unlikely that the indentation will impact the bottom of the PDMS substrate. It would be reasonable to conclude that this area is therefore not worth modelling. Finding the balance point at which this parameter stops impacting results can decrease the computational power needed for the simulation without compromising the results' usefulness. This was chosen as the main focus of this specific investigation.

Choosing a parameter to focus on first is a challenge since improvements in one area may cause changes in another. Therefore, basic checks were ran using the final parameters to ensure the consistency of these optimisations. All tests in this section were completed at a force of 1000nN. Ideally, this would be repeated across a range of forces to validate the refinements' accuracy; however, this was not possible within the given time. By choosing the maximum experimental force for this testing, I aimed to maximise the differences in results and test the features at maximum deformation. This section will be primarily descriptive; however, the final chosen parameters are summarised in Table 1. This section details two major refinements - the geometry dimensions and meshing. However, other parameters were also refined in this investigation, but these were summarised in the previous section for brevity.

2.2.1 Geometry Dimensions

While the geometry was simplified in the basic model setup, further improvements can be made. As mentioned, the experimental and literature values for the dimensions of the lab setup are excessive for the nanoscale testing used here. Therefore, three parameters were chosen for optimisation - the

PDMS depth, width and PDMS/fibril length. This is clarified in Figure 7.

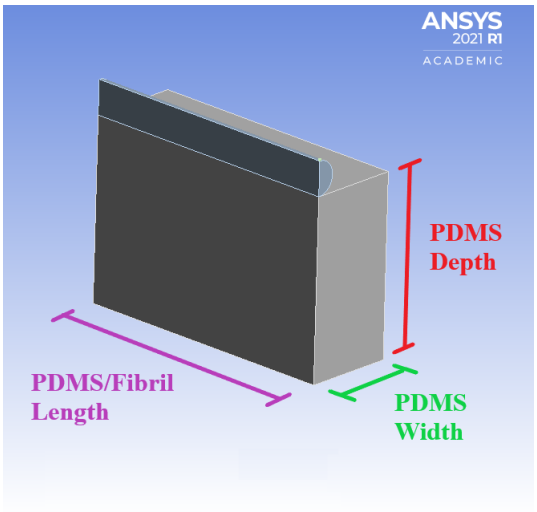

Figure 7 *A labelled screenshot identifying the three dimensions refined in this section.*

A reasonable testing range - based on the existing data and initial testing - were chosen to see at which point increasing the parameter stopped impacting the results. Then the simulation was run at the varying PDMS depths while keeping all other variables constant. The resulting tip displacements were recorded. Figure 8 highlights how adjusting the PDMS depth across a range of values impacts the resulting tip displacement.

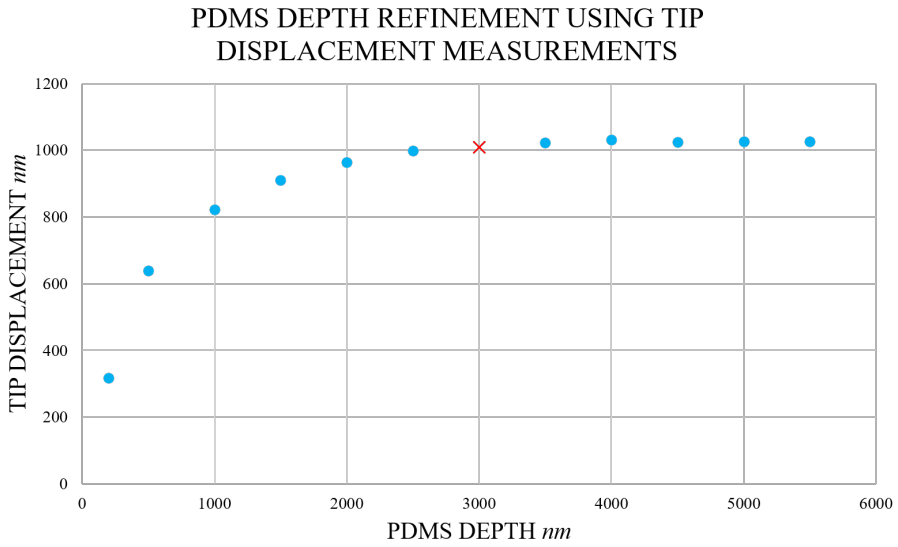

Figure 8 *A plot showing the resulting tip displacements after varying only the depth of the PDMS. The cross indicates the chosen value for the final simulation.*

A clear trend can be seen in these results. At small depths, increasing the PDMS depth increases the proximity to the experimental tip displacement of 1116nm, but after a certain point levels off. This asymptote is key to the optimisation process. By choosing a point that is close to the asymptotic value while minimising the PDMS depth, fewer FEA elements are used to model the PDMS surface - without impacting the accuracy of the result. It was important to use this visual trend instead of just choosing the results closest to the experimental value. Changing other parameters afterwards may change the individual values of this plot but do not change the trend. Extreme values (i.e. large PDMS depths) were also checked to see if these results were reliable; however, meshing restrictions made these results less accurate. Graphs showing the other refinements can be found in Appendix B, and these tests were carried out similarly. The final chosen parameters can be found in Table 1.

2.2.2 Nodes & Meshing

A key aspect of FEA is meshing. Ansys can automatically generate a mesh, but the power to improve the accuracy of a simulation comes with refining

this mesh. Although, it is interesting to note that even this basic mesh provided surprisingly valuable results. There are likely infinite ways to refine the meshing, all of which could not be explored in the given time. The limiting factor in this investigation is the student Ansys programme used, which places a limit of 128K nodes/elements on structural analysis models [21]. While this number is large, it still placed restraints on the meshing used. The first improvement made was the method of meshing. The mesh was upgraded to the tetrahedral method to increase the quality of the mesh. Due to the complex nature of mesh refinement, specific data on these tests are not provided in this report.

The main improvements were focused on basic refinements that allowed the user to set the mesh densities at specific points in the geometry. These are called 'sizing' features. Here, vertex and edge sizing were considered as they were found to be most relevant in initial testing. As shown below in Figure 9, the edge sizing method set the element size along a chosen edge. The vertex method involved a 'sphere of influence' where the user can select the element size within a set radius of a point.

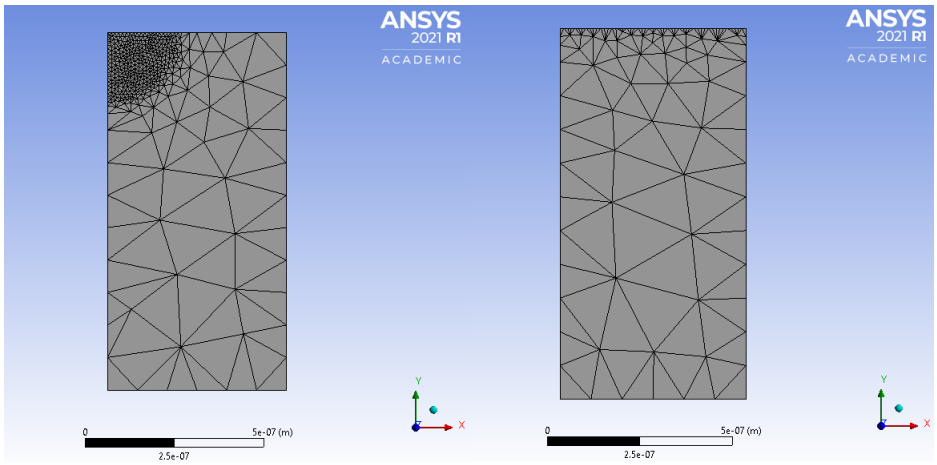

Figure 9 *Screenshots comparing vertex sizing on the top left corner (left) and edge sizing on the top edge (right) of a cuboid.*

The primary consideration while creating this mesh was the properties to be measured. It makes the most sense to increase the mesh density around the critical points of the simulation for increased accuracy. These were identified as under the tip and between the fibril and the substrate for the tip

displacement and bending measurements.

Tip Meshing Refinement

For accurate measurement of the indentation displacement, the meshing on the fibril should be refined. Due to the spherical geometry of the tip, it seemed most appropriate to refine the mesh beneath the tip using the vertex sizing refinement. Basic testing showed that other meshing methods on edges around the fibril were not as effective as this. While setting a high radius for the 'sphere of influence' may seem appropriate, it was found that the cylindrical shape of the fibril made this meshing less smooth. Surprisingly, the optimum was found to be just above the radius of the tip. In terms of the element sizing, the small size of the tip meant an element size of 2nm was found to be most appropriate. Previous meshes gave element sizes that were bigger than the tip itself. This element size is very small but was possible due to the small 'sphere of influence'.

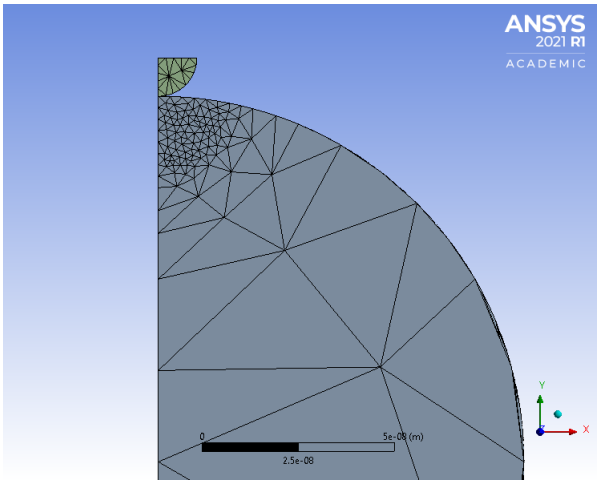

Figure 10 *A screenshot showing the vertex sizing used under the point of the tip.*

Bending Meshing Refinement

Refining the mesh for the bending measurement proved a more challenging task. When considering the tip displacement measurements, there are

experimental data for comparison. However, the purpose of this investigation is to gain insight into the bending. Therefore, a balance had to be found between a convincing mesh and keeping results as accurate to the experimental tip displacement as possible.

During initial testing, it was found that symmetry was key to an even - and therefore accurate - mesh. It was not found that specific refinements on the PDMS at the point of maximum bending were more effective. It is visible in Figure 9 that the vertex meshing at a corner created less even meshing in the rest of the object than the edge meshing. While putting a spherical vertex mesh under the PDMS below the tip seemed logical, it was found to cause irregularities in the meshing along the length of the fibril. Though the bending of the PDMS below the tip is important, bending along the fibril length also contributes significantly to the displacements. Therefore, an edge refinement (of element size 10nm) was used along the fibril's length for both the PDMS and fibril meshing, as shown below.

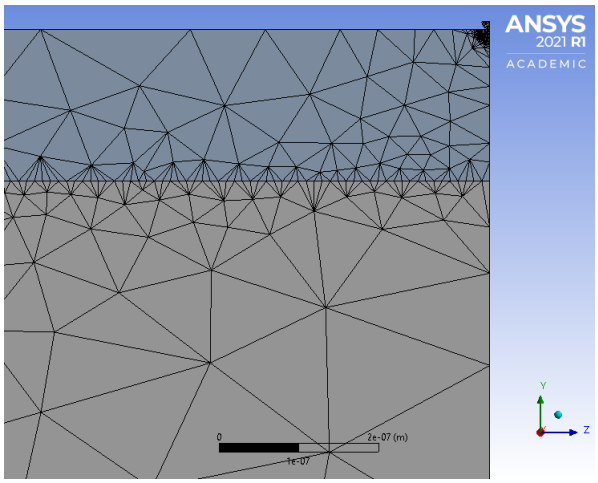

Figure 11 *A screenshot showing a side view of the geometry with edge refinement along the bottom edge of the fibril and top edge of the PDMS.*

This investigation believes that this meshing along the fibril's length provides more accurate bending data than if the mesh were focused only beneath the tip. As shown in Figure 12, edge refinements were added to two additional edges of the PDMS. It provides a similar focus around the tip as a sphere of influence to gain more valuable data on the bending displacement. However, this investigation believes the increase in mesh symmetry and smoothness compared to vertex sizing provided more accurate results.

2.3 Model Comparison

Figure 12 shows the model before and after optimisation. There are clear differences in the sizing and mesh density. The original mesh used 8839 nodes whereas the improved mesh used 71503. More elements in the future could refine the mesh even further. Clearer images of the final geometry and meshing can be found in Appendix C.

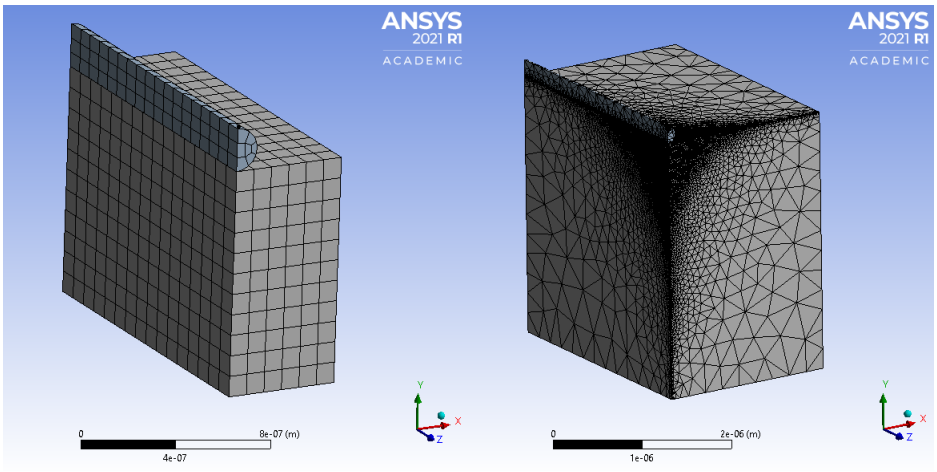

Figure 12 *A comparison between a) the model before any manual refinement and b) the final model after the refinements detailed above.*

2.4 Final Investigation Setup

A summary of the simulation setup used in this investigation can be found here. Table 1 shows a summary of the final parameters used, which are a combination of experimental data, optimised parameters and suggested values.

	PDMS	Collagen Fibril	Tip
Young's Modulus *GPa*	0.0037	5	179
Poisson Ratio	0.49999	0.47	0.3
Dimensions *nm*	depth = 3000 length = 3000 width = 2000	diameter = 189.9 length = 3000	radius = 10

Table 1 *A summary of the final parameters used in this investigation.*

To summarise, using an Ansys simulation this investigation aims to

- simulate a tip displacement as close to the experimental results as possible, while staying true to the experimental setup
- find a relationship between the proportion of bending and indenting comprising the overall tip displacement
- optimise the parameters to increase the accuracy and usefulness of the simulation.

This will be achieved by using point displacement probes to measure two variables - the **total tip displacement** and the **maximum displacement of the PDMS** a.k.a. the bending displacement. The tip displacement shall be compared to the experimental values and the indentation will be calculated as

Indentation Displacement (nm) = Tip Displacement (nm) - Bending Displacement (nm).

The ratio between the indentation and bending displacements as a proportion of the overall tip displacement shall be found. The forces tested shall be the same as used for the experimental data however, for use in the simulation they will be inputted as a quarter of their value.

3 Results

Below are the results obtained during the course of this investigation. The data shown here is a summary of the key results, please refer to Appendix D for the full data.

3.1 Comparison to Experimental Results

A key factor in determining the usefulness of the model is how well experimental results were recreated. Figure 13 shows the experimentally obtained tip displacements and the tip displacements obtained from the simulation at each force.

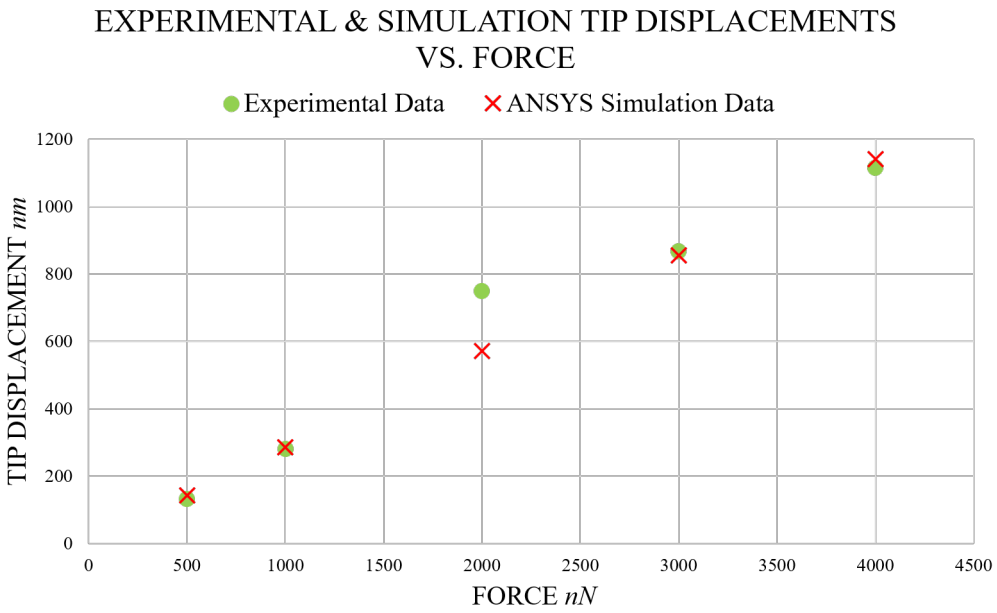

Figure 13 *A graph showing the final results - a comparison between the experimental and simulation tip displacements at each force. Note that marker sizes have been exaggerated for visibility and error bars on the experimental data are too small to be seen.*

As is clear from this graph, the simulation can be considered successful in many ways. If the experimental result at 2000nN is viewed as an outlier, the simulation has closely replicated the linear relationship between the tip displacement and force. The full data found in Appendix D shows the

percentage differences between the experimental and simulation tip displacements are less than 5% (except the 2000nN point). There are no given errors for the simulation results. The error in the results is difficult to quantify since the simulation provides the same results on each run. However, some uncertainties and simplifications reduce the precision of the results in other ways.

While only one point falls within the provided experimental uncertainty of 4nm, it is reasonable to suggest there may be factors not accounted for by this figure. For example, some parameters provided were either estimates or literature values (instead of actual data from the original setup). Additionally, the provided error was taken from the AFM but did not include other errors that may have occurred throughout testing. An example could be testing on both O and G regions of the fibril without distinction. Collecting more data about the experimental setup and better quantifying the errors may allow for a more accurate simulation in the future.

3.2 Bending vs Indenting

The graph below details the most important results of this project - modelling how much of the tip displacement is due to the fibril and PDMS substrate bending versus the fibril indenting.

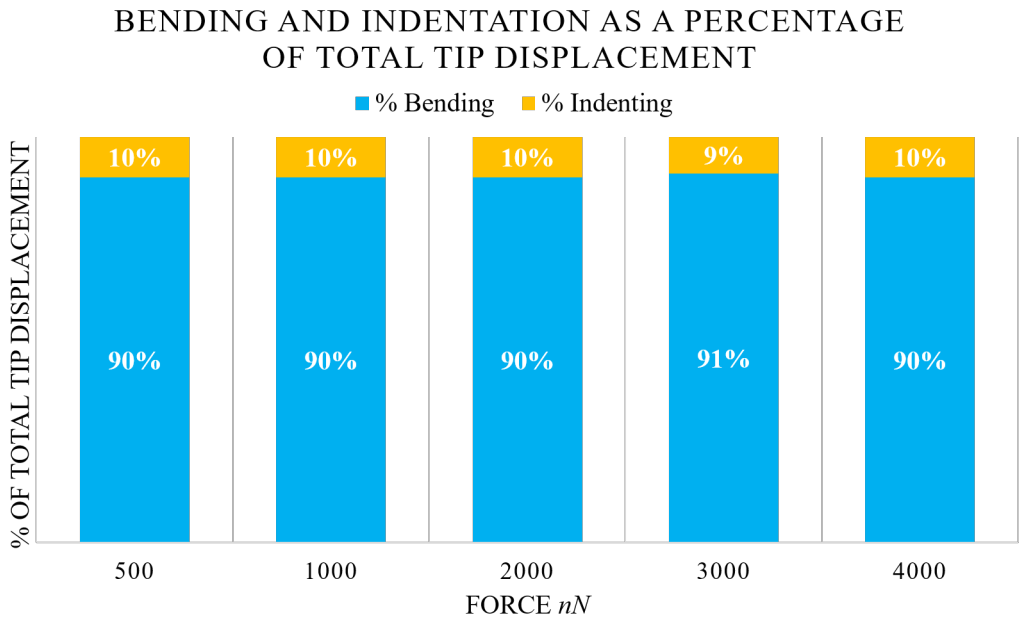

Figure 14 *A chart showing the percentage of bending and indenting as a proportion of the total tip displacement at each tested force. Percentages given to 2 significant figures.*

These results show a strong ratio of approximately 90% bending to 10% indenting occurring in the simulation. The uncertainty in the accuracy of the previous results makes interpreting these results difficult. However, the broadly consistent ratio across the forces is reassuring, and it is clear that bending plays a very significant role in this scenario. This is consistent with the material properties of PDMS- its relatively low Young's modulus means PDMS is more likely to deform under load than a material with a higher Young's modulus. Changing the surface material was not tested in this investigation but should be explored in the future. Further research could also use more data (or another software) to check whether this specific ratio is an artefact of the FEA method used or an accurate representation. The implications of these results are considered in the Discussion section.

3.3 Parameter Optimisation Results

The simulation was run for each force before and after the optimisation improvements detailed in Section 2. The initial parameters were either random

or automatically generated by Ansys. This increase in accuracy is merely intended to illustrate the power of refining a simulation. Quantitative measurements depend on how close the original parameters happened to be to the optimum value, and, of course, there are many more improvements that could be made. However, on average, there was a 33 % percent improvement in the accuracy of the results compared to the experimental values. More details can be found in Appendix D.

There are many other parameters that would benefit from this type of testing. For example, finding the most accurate Young's modulus or Poisson ratio where these values were only estimated. However, it should be noted that more data on the true experimental setup would further improve the accuracy and reliability of the results.

3.4 Result Summary

While interpreting the results of this investigation is not straightforward, there is certainly value to be found. Find below a summary of the key results and the main conclusions from this project.

- Ansys created a reasonably accurate representation of the experiment in the time given. On average, there was an 6% deviation from the experimental values (which includes a potential outlier result). To further verify the accuracy of this model, more detailed experimental data and errors would be needed.

- There was a well-established ratio of bending to indenting - 90%:10%. This suggests that the bending of the PDMS substrate makes up a significantly larger portion of the experimental tip displacement than the indentation into the collagen fibril itself.

- Optimising the parameters of this model made a big improvement in the accuracy of the results - on average a 33% improvement. This shows that simple steps can be taken to reduce unnecessary modelling features in a simulation to make way for more accurate results.

4 Discussion

The most significant finding in this investigation is the ratio of bending to indenting shown in the model - and how the bending dominates as a proportion of the tip displacement. The value of this finding varies depending on the application of the original results, but it is undoubtedly informative.

Consider the indentation hardness methods discussed in the introduction. The hardness of a sample can be calculated using the applied force and the area of the impression left behind. For nanoindentation, this area is too small to measure by eye and to use electron microscopes to image the area would be time and resource consuming. However, the results from this investigation suggest we can calculate values for the true indentation depth from the experimental tip displacement using a ratio. If the tip geometry is known, the area of an indentation impression could be calculated from this indentation height - and hence, the hardness can be found. Therefore, the results from this investigation can directly inform calculations and provide insight into the mechanical properties of materials. This could be extended to other methods used to calculate material properties from nanoindentation data.

Greater insight into the properties of collagen increases its usefulness within tissue engineering, as highlighted in the introduction. The results found in this investigation could inspire future experimental design that accurately and precisely details the mechanical properties of collagen. This increases its viability as a scaffolding material in creating artificial organs. Furthermore, better methods could be used to test specific types of fibril against criteria for desired scaffolding (and hence tissue) properties.

While this area of research is relatively new, there is much to be learnt from the existing literature. This can inform future directions of work and provide ideas for applications of this investigation. There are other investigations of the mechanical properties of collagen - some using AFM nanoindentation and others using methods such as stretching or bending the fibril [8] [18] [20]. Both of these would be interesting to model in a similar way to this investigation. A key takeaway is how computer simulation work can be used to guide and explain experimental results and vice versa.

As discussed throughout this report, there is much room for improvement on the work described here, and hopefully, this model provides a good foundation. A better understanding of the experimental setup - including more specific numbers for the material properties used - would increase the accu-

racy and reliability of the simulation. If this is not possible, then perhaps a method of optimisation like that of Section 2.2 could be used to find values for the Young's moduli or Poisson ratios that give the best results.

The mechanical properties of collagen could be explored in a different way. For example, nanoindentation will often be used to create a force-displacement curve by recording the displacement throughout the time of a loading and unloading process. This could be achieved using this model by time-stepping the force over a certain number of seconds and recording the displacement at each point. A force-displacement curve can provide a "mechanical fingerprint" of a material's response to deformation. For example, the slope of an unloading curve can indicate the stiffness of a material, which can be used to calculate a value for the reduced Young's modulus [14].

More examples of future work include using a higher node count to refine the mesh further. This could allow for the complexity of the collagen fibril to be modelled more accurately and potentially lead to better results. The OG region structure could be modelled, or even the helical structure of the collagen molecules themselves [7]. It would also be interesting to explore non-linear analysis, which is possible in Ansys. Perhaps the complex nature of the collagen fibril could be better modelled using non-constant values for the mechanical properties. Looking further afield, this model could be applied to other biological materials undergoing nanoindentation testing. There could be potential for a simulation, calibrated using existing experimental data, that could simulate indentation testing on materials that cannot be tested using in a lab. This speaks to the surprising value of a simple computer simulation.

There are many different avenues for future exploration in this topic, which shows the vast potential for discovery within biophysics.

5 A Summary for the General Public

Collagen is an essential protein found everywhere in the human (and animal!) body - from our flexible muscles to our rigid bones. It's so integral to how our bodies work that we can even use it to build new body parts. With a few other ingredients, collagen could provide the structure to grow new organs from a few cells. A fascinating idea!

To achieve this, we need to understand more about what collagen is like - particularly how strong it is. This will tell scientists the best ways to use it to get the results we want. Engineers have many different methods of testing how strong materials are - which is why we trust buildings and bridges to stay upright! However, science hasn't done as much testing on materials as small as collagen. Collagen can be imagined as a tiny cylinder that comes in strands, much like hair. But unlike hair, collagen is so small that we can't see it. To figure out exactly how strong collagen is, scientists needed to find a test that could work on things too small to hold or see. This is because the traditional methods that engineers use often rely on taking measurements by eye.

One way to test this strength is a method called AFM nanoindentation, which involves pressing a tiny hard tip into a strand of collagen. By seeing how far it can push into the strand with a certain amount of force, we can measure how 'strong' the material is - even if we can't see it.

Imagine the difference between poking a finger into a loaf of bread compared to a block of hard cheese. We can tell that the cheese is 'harder' than the bread because of how much less our finger would move. If we wanted to, we could probably measure with a ruler how far our finger goes into the loaf or the cheese when we poke it. Then we could say: the further our finger moves, the softer the object must be. And if it doesn't push very far, the object must be hard. This matches what we experience when we hold bread or cheese in our hands (or mouths!). This idea is similar to how we can test the strength of collagen using AFM nanoindentation. Our finger is the tiny hard tip, and the collagen is the bread or cheese we press into.

If we thought about it more, we'd realise that how far our finger moved not only depends on the object (or food item) we poke, but also the surface we've placed the object on for our test. After all, it's not possible to press into an object floating in midair. Imagine pressing a finger into a block of cheddar cheese resting on a solid kitchen surface. Then, imagine resting the block of cheese on a soft mattress instead (a strange concept!) to repeat the

test. Our finger would likely move more in a test on a mattress compared to a kitchen counter. Even if the cheese doesn't move much when we press into it, the mattress beneath it will! Whereas a solid kitchen surface won't bend beneath the cheese, even with a lot of force!

If we only relied on how much our finger moved during these tests to tell how strong the object was, we would get a different answer for the counter and the mattress. Our finger would move more if we poked the cheese on a mattress and would move less if we poked the cheese on a hard surface instead. But nothing about the strength of the cheese itself has changed. Luckily, we would see that the mattress would cave in as we pressed into the cheese. But collagen strands are just so small that even our best microscopes cannot let us see what is causing our tip to move by a certain amount - whether the tip is indenting into the collagen strand or the surface beneath it is bending out of the way.

This investigation aimed to determine whether the tip is digging into the collagen during AFM nanoindentation or just bending the surface beneath it. In this project, we created a computer simulation of the experiment. This model gives us very similar results to the real thing - with the advantage that we can see what is going on. When designing a computer model, there are many considerations to make since it's impossible to recreate reality exactly. But these limitations don't mean that we can't still get helpful information from simulations.

We found that, when resting collagen on a substrate called PDMS, the tip both indents into the collagen strand AND causes the PDMS surface beneath the collagen to bend. In fact, the majority of the distance the indenter tip moves is due to the PDMS surface bending. This tells us that it's not straightforward to interpret the experimental results when we can't see what's going on. When we use this method to test the strength of collagen, we have to know that our results depend on what surface we've placed it on for testing. We now have a specific ratio from the simulation of how much bending happens. This means scientists can use this knowledge when designing new experiments and interpreting their results. More specifically, they know they cannot rely only on how far the testing tip moves to determine how strong a material is.

The more accurate results we can get about the strength of collagen, the more successful we will be when designing new organs. While tests like this can seem abstract, they can lead to advancements in our understanding of the human body, which could ultimately save lives.

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A Data from the Lab Experiment

Find below a summary of the information provided by Emilie Gachon from her AFM nanoindentation experiment in the lab.

Force *nN*	**Tip Displacement** *nm*
4000	1116 ± 4
3000	867.7 ± 4
2000	750.5 ± 4
1000	281.4 ± 4
500	132.86 ± 4

Table A1 *A summary of the experimental AFM results showing the tip displacement for a given force.*

Experimental Set-Up

- Tip Type: "Budget Sensors' Monolithic Silicon Tap300-G" tips
- Collagen Type: Type I collagen
- Collagen Source: Adult rat tail tendon

Parameter	**Value**
Tip Radius	10nm
Fibril Diameter	189.8nm
PDMS Thickness	1mm
Estimated Tip Young's Modulus	179GPa
Estimated Collagen Young's Modulus	5GPa

Table A2 *A summary of the material properties and dimensions from the lab set-up.*

B Geometry Refinements

The remaining graphs from the geometry dimension optimisation testing can be found here. The crosses indicate the chosen value for the final simulation.

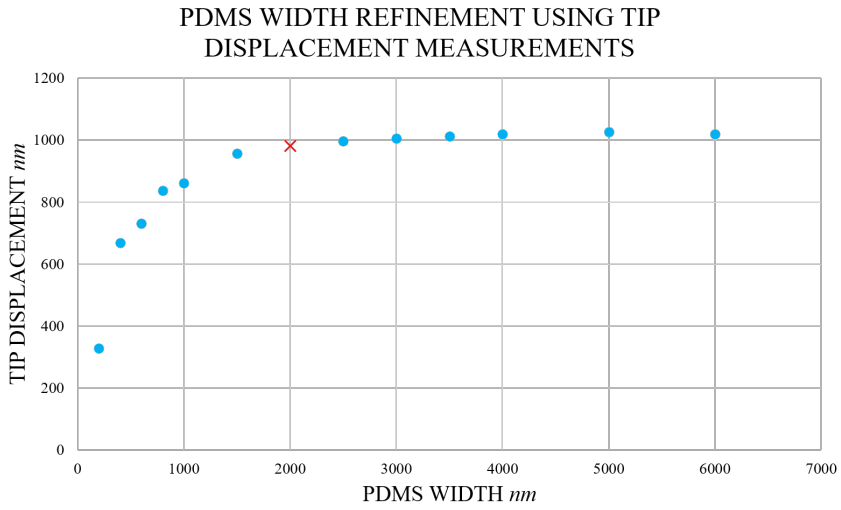

Figure B1 *A plot showing the resulting tip displacements after varying only the PDMS width.*

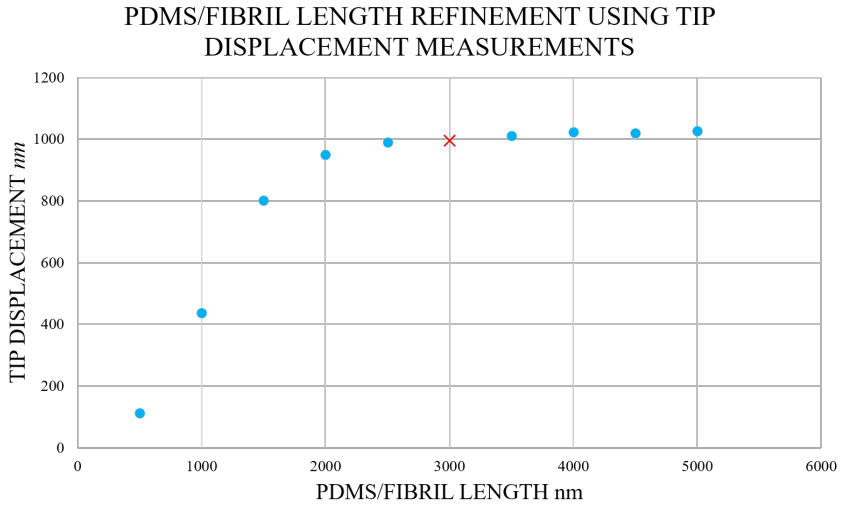

Figure B2 *A plot showing the resulting tip displacements after varying only the PDMS/fibril length.*

C Additional Model Images

Additional images of the final geometry and meshing can be found here for clarity.

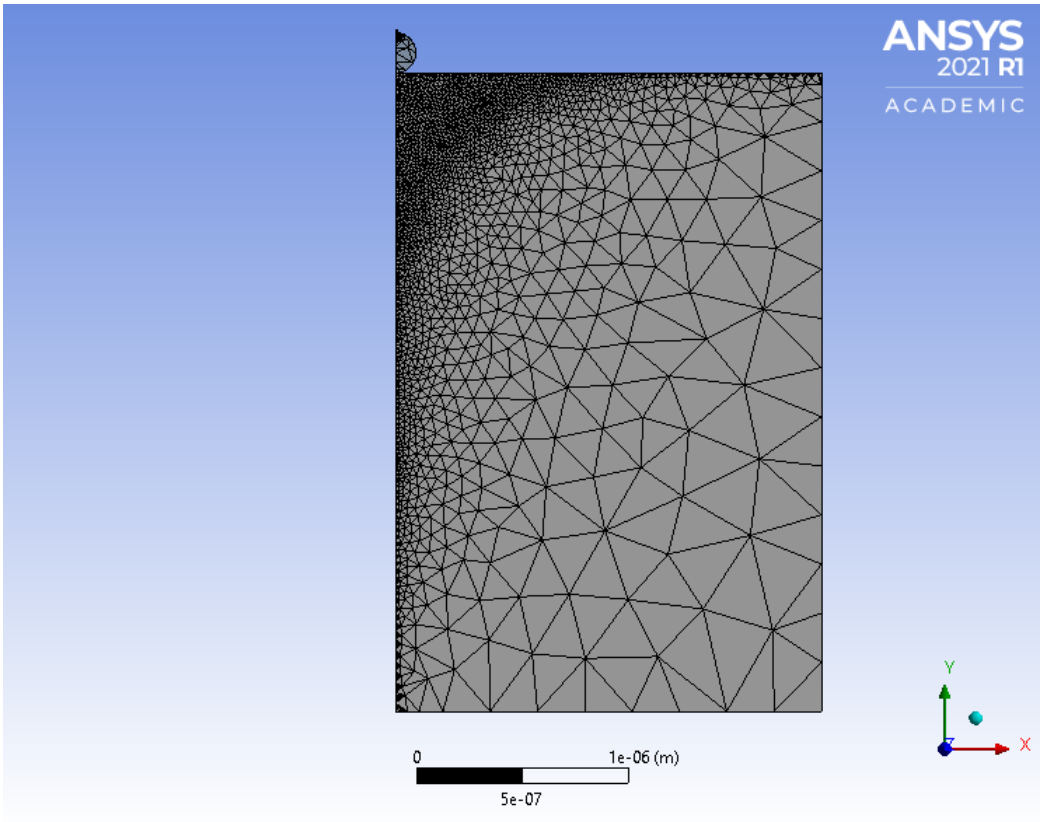

Figure C1 *A screenshot showing the front of the geometry and meshing.*

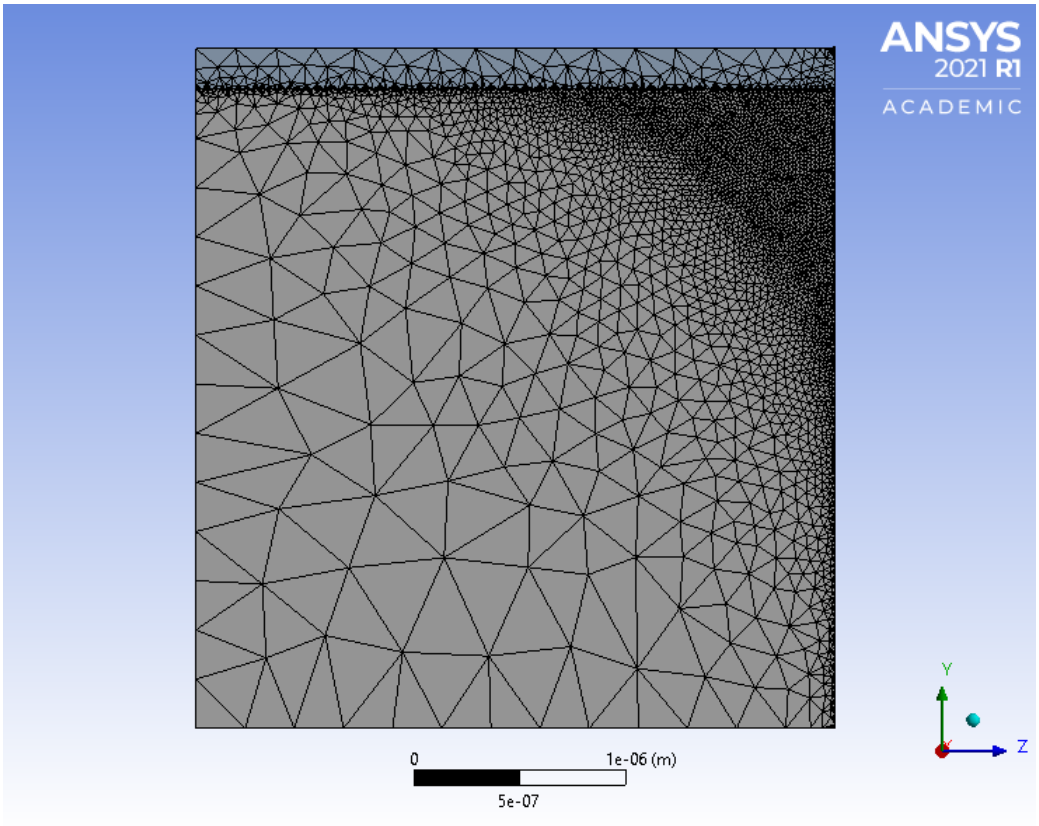

Figure C2 *A screenshot showing the geometry and meshing along the fibril length.*

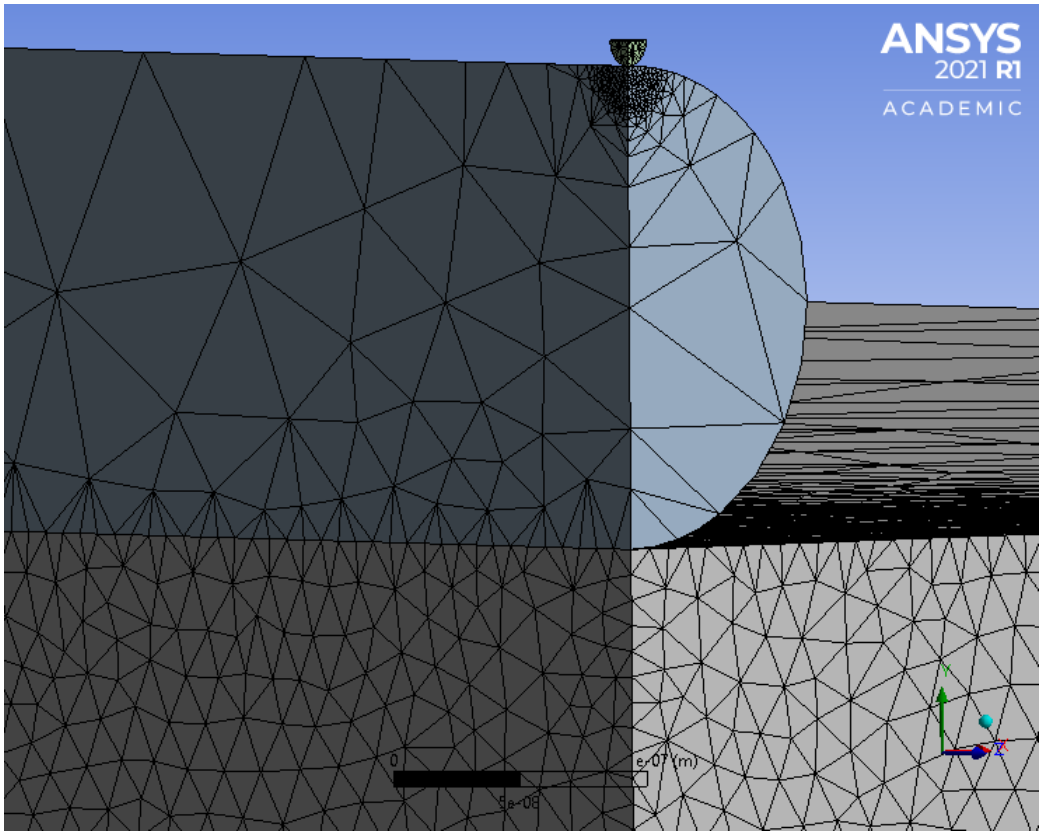

Figure C3 *A screenshot showing a closeup of the geometry and meshing.*

D Results in Full

Find here the results in full for future reference. This includes the percentage difference between the experimental and simulated tip displacements and details of the parameter optimisation results.The results are given to three significant figures based on the precision and certainty in the parameters used in this model. Percentages are rounded to the nearest percent.

Force nN	Experimental Tip Displacement nm	Simulation Tip Displacement nm	Percentage Difference %
4000	1116 ± 4	1140	2
3000	867.7 ± 4	856	1
2000	750.5 ± 4	571	23
1000	281.4 ± 4	285	0
500	132.86 ± 4	143	5

Table D1 *A table containing the final simulation results in full, alongside the experimental values and a percentage difference calculation .*

Note: the percentage differences given above are calculated with respect to the maximum and minimum experimental values defined by the given errors.

Force nN	Tip Displacement Pre-Refinement nm	Tip Displacement Post-Refinement nm	Percentage Improvement %
4000	704	1140	35
3000	528	856	38
2000	352	571	29
1000	176	285	36
500	88.0	143	26

Table D2 *A table comparing the tip displacements obtained before and after optimising the model. The percentage improvement is the increase in the accuracy of the results in proportion the the expected value.*