Comparison between the Elastic Properties of Balloons and of Exosomes

Tilde Berglind
berglindtilde@gmail.com

under the direction of
Prof. Dhrubaditya Mitra
Nordita

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Abstract

The protein profile of exosomes, a subgroup of extracellular vesicles, influence their elasticity. If the elastic properties of the exosomes were to be measured, they could be used for identifying the protein profile of the surface. The aim of this study was to investigate whether balloons are suitable for modelling the elastic properties of exosomes on a macroscopic scale. This was done by calculating the spring constants of balloons with different pressures, and then comparing the result with the theory behind the AFM - the Hertz model. The study showed that balloons are not suitable models for exosomes, since the Hertz model was not applicable to balloons.

Acknowledgements

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Contents

1 Introduction

Extracellular Vesicles (EV) are nanoparticles comprised of a lipid bilayer membrane surrounding internal DNA, RNA, protein and lipid cargo. Most cells secrete EVs as part of their normal physiology, and they are important mediators for intercellular communication, both locally and in distant tissues [1]. Over the last decade, EVs have been the subject of intensive research and the area has seen an increase in the understanding of their function. Over 4000 research articles have been published regarding these vesicles, and the global EV-based diagnostics and therapeutics market is projected to reach 264 million USD by the year 2024 [2].

1.1 Exosomes

Exosomes are a subclass of EVs secreted from normal and diseased cells, and that can affect the function and behaviour of target cells [3]. The lipid bilayer membrane of exosomes is composed of signal proteins, lipid miRNAS and mRNA. They provide autocrine, paracrine and endocrine signals to cells through the transfer of their contents. Autocrine signals are local and signals to the same cell type, leading to changes in the cell, paracrine signals are local signals and used in order to communicate between different cell types over short distances and, lastly, endocrine signals are signals sent over long distances between all types of cells, usually carried in bodily fluids, such as blood. Exosomes release their content into the target cell by fusing with the plasma membrane of the target cell. [1].

1.1.1 The Importance of Exosomes in Medical Science

Exosomes are involved in many processes in the body. They are present in all bodily fluids, including plasma, urine and saliva and are used for communication within the tumor microenvironment. The cancer cells communicate with each other through the secrete and delivery of miRNA that are packed into tumor-released (TR) exosomes. The understanding of the role and behaviour of miRNAs secreted by TR exosomes is crucial in the field of cancer biomarker discovery, and for the development of new biomedical applications for cancer therapeutics. Additionally, exosomes can be used to develop antiviral or vaccine treatments since they can limit infections. However, the research on exosomes are still in an early stage, and more studies are required in order to fully understand the relation between exosomes and viruses, and to be able to develop new strategies to interfere with disease development. [4, 5]

1.2 Previous Research

Many studies have been made regarding exosomes. One of the studies made propose an explicit connection between surface proteins of exosomes and their elasticity. If the elastic properties of the exosomes were known, they could be connected to the amount of proteins on their surface. By utilizing this knowledge, the exosomes could be manipulated and used in medicine. [6]

1.3 Background

Elasticity is the ability of a material to deform to a defined extent in response to a force, and then return to its original state when the force is removed. The elasticity of a material can be described by Young's modulus, and it is the most relevant modulus that can be applied to exosomes. Young's modulus describes the stiffness of a material, meaning how easily a material is bended or stretched. [2]

Young's modulus can be calculated using the Hertz model, which is a classical theory of contact mechanics. Contact mechanics is the study of the deformation of solids that touch each other at one or more points. Hertz was the one who solved the contact problem of two elastic bodies with curved surfaces. The theory of contact between elastic bodies can be used to find contact areas and indentation depths for simple geometries. Hertz contact stress refers to the localized stresses that develop as two curved surfaces come in contact and deform slightly under the imposed loads. The amount of deformation is dependent on the modulus of elasticity of the material in contact. Furthermore, the model assumes that there is no friction between the contacting surfaces, absolute elastic behaviour and homogeneity for the sample tested. [2, 7]

1.4 AFM - Atomic Force Microscopy

Atomic force microscopy (AFM) can be used in order to apply a controlled force onto a sample. It consists of a cantilever, see Figure 1, that probes the surface of a sample at a defined speed with a tip, and thereby apply a force onto it. The tip is usually called the indenter. The forces that arise between the tip and the surface of the sample cause the cantilever to bend. A laser is aimed at the back of the arm, from which it is reflected towards a photodetector. The photodetector converts light into an electrical signals. [8]

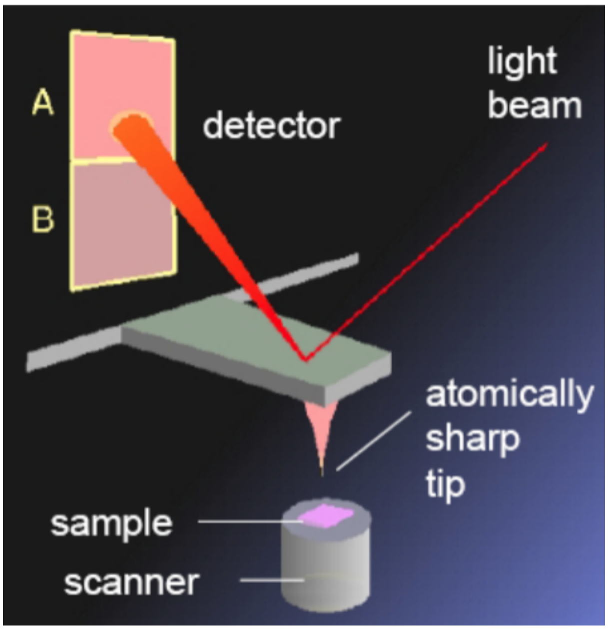


Figure 1: A schematic representation of an AFM microscope. A sharp tip is attached to a cantilever that can probe the surface of the sample observed. When sufficiently close to the sample, the tip is affected by forces at the sample surface. This causes the tip to move, which in turn causes the entire cantilever to bend. The cantilever bending is detected optically by a light beam and a photodetector. The scanner is used to move the sample under and in proximity of the tip. [8]

One of the different modes of operation for AFM is called contact mode. In contact mode, the tip of the cantilever has full contact with the sample throughout the whole measurement. As the tip scans the surface of the sample and passes over peaks and valleys, moving up and down with the contour of the surface, the cantilever is bent and twisted. When the tip passes these height differences, it changes the angle of the beam hitting the photodetector. By scanning the surface as described, a computer can generate a three-dimensional map of the topography of the surface, which thereafter can be used to interpret the structures of exosomes under varying forces. Furthermore, the computer allows an analysis of the data by plotting the force as a function of the indentation of the sample, where several mechanical parameters can be extracted. [9, 6]

1.4.1 Mechanical Properties of AFM

Mechanical properties of a sample can be measured through utilizing AFM. Additionally, by plotting a force–indentation curve using a computer connected to the AFM, several mechanical parameters can be extracted. For instance, Young's modulus can be obtained by fitting an indentation model to the approach curve, or as previously mentioned, be calculated using the Hertz model. The Hertz model is the most frequently used model of indentation used to determine the biomechanical properties of EVs by AFM. As earlier discussed, Hertz contact stress refers to local stress that develop when two surfaces come in contact with each other, which can be compared to when the AFM probe the exosomes. [2]

There are different equations of the Hertz model, depending on the geometry of the indenter. For a parabolic indenter, the force is proportional to the indentation to the power of 3/2, and the constant of proportionality involves the radius of the indenter, Young's modulus and Poisson's ratio - which for EVs is known to be 0.5. This is shown in equation (1), where the F is the force, R^t the radius of the tip, E Young's modulus, v

the Poisson's ratio and δ the indentation. [2, 7]

$$F(\delta) = \frac{4\sqrt{R_t}}{3} \frac{E}{1 - v^2} \delta^{3/2} \tag{1}$$

The parabolic model is usually used if the indenter is a sphere as well, even though it exists a specific equation for spheres. This is because the parabolic model is easier to fit to the force-indentation curves.

1.5 Hooke's Law

Hooke's law states that the force needed to extend or compress a spring by some distance is proportional to that distance. Mathematically, Hooke's law and the force F applied to a spring is given by

$$F = kx \tag{2}$$

where k is the spring constant and x is the displacement of the spring. [10]

In Figure 2, the lower spring represents the balloon, and the upper spring the spring pushing against the balloon. When force is applied to the upper spring, it compresses and extends, meanwhile it pushes down on the lower spring, which can be observed in Figure 2b.

The upper spring is compressed by Δl, which is the length it gets compressed when the force is applied, and extended by $(L - z)$. L is the initial length of the lower spring and z is the length after the force has been applied. The total length change of the lower, compressed spring can be expressed as $(L - z)$. The total length change of the upper spring is its extension minus its compression. The force required to compress and extend the upper spring can be given by using Hooke's law, seen in equation (2), and can be expressed as

$$F = k((L - z) - \Delta l) \tag{3}$$

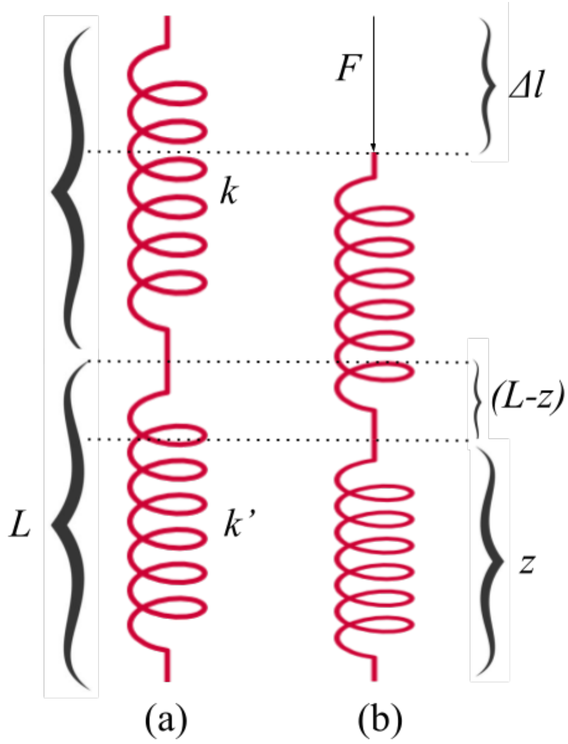


Figure 2: A system of two springs. (a) shows a system where the springs are at rest, (b) shows a system where a force has been applied. In (b), the upper spring gets compressed by Δl and extended by $(L - z)$, due to the force F, and the lower spring gets compressed by $(L - z)$.

where k is the spring constant of the upper spring, and $((L - z) - \Delta l)$ is the total length change of the upper spring. However, the compression of the lower spring is of interest, which can be given by solving for $(L - z)$. Mathematically, this is given by

$$F = k((L - z) - \Delta l) \Leftrightarrow \frac{F}{k} + \Delta l = (L - z) \tag{4}$$

where F, k and Δl can be measured. However, since it can't be assumed that the relation between the force applied to the balloon and its displacement is linear, a unknown number d will be added as an exponent of the displacement of the spring, which can be seen in equation

$$F = k'(L - z)^d \tag{5}$$

where F is the force, $(L - z)$ the displacement of the balloon, k' the spring constant of the balloon and d an unknown number.

Thereafter, in order to determine the exponent d in the relation between the force of the balloon and the displacement, i.e. determine whether d is 1 or not, logarithms will be used, seen in equation

$$F \propto k'(L-z)^d \Leftrightarrow \log_{10}(F) = d\log_{10}(L-z) + \log_{10}(k') \tag{6}$$

where the slope of $\log_{10}(F)$ is exactly d, and can be found by linear regression. If the relation between F and $(L-z)$ turned out to be proportional, the spring constant of the balloon could be given by utilizing Hooke's law, seen in equation (2). The spring constant of the balloon can then be given by the equation

$$F = k'(L-z) \tag{7}$$

where k' is the spring constant of the balloon, F the force acting upon the whole system of the balloon and the spring, and $(L-z)$ the displacement of the balloon. This mathematical expression is given through utilizing Hooke's law, seen in equation (2).

The force applied on the system is the force of gravity and can be calculated using Newton's equation, seen in equation

$$F = mg \tag{8}$$

where F stands for the force (N), m the mass (kg) and g the gravitational acceleration, $9.82\,(\mathrm{m/s^2})$ [11].

1.6 Aim of Study

This study aimed to examine the suitability of balloons as models for exosomes, by calculating the spring constants of different balloons and thereafter seeing whether the Hertz model is applicable to balloons, which is a necessary condition for using balloons as models for exosomes.

2 Method

Balloons with different pressures were poked with tips of different shapes, a sharp tip and a dull tip. The balloons were used in order to model the elastic properties of exosomes on a macroscopic scale, where the exosomes were emulated by balloons and the cantilever by a spring with a tip.

2.1 Measurement of the Spring Constant

With the aim of measuring the spring constants of different balloons, the spring constant of the spring first had to be measured. It was measured by using a setup like the one shown in Figure 3.

In the setup used, a tube with a spring and a cubic tip was attached to a rod, which in turn was attached to a movable platform. However, only half of the tip was inside of the tube, the other half rested upon the scale below. The front of the platform and the rod attached to it was moved downwards by the turning of an arm. As the rod was pushed down, the spring was compressed and thereby pushed the cubic tip against the scale. Subsequently, as the rod was pressed down more, the spring got compressed more causing the cubic tip to get more and more pushed into the tube. The rod was pushed down by 0.5 millimeters at a time, which was controlled by observing the arm that had a millimeter scale on it. In total, the rod was pressed down by 4 millimeters until the tip was fully inside the tube, and the experiment was performed twice. The arm measured

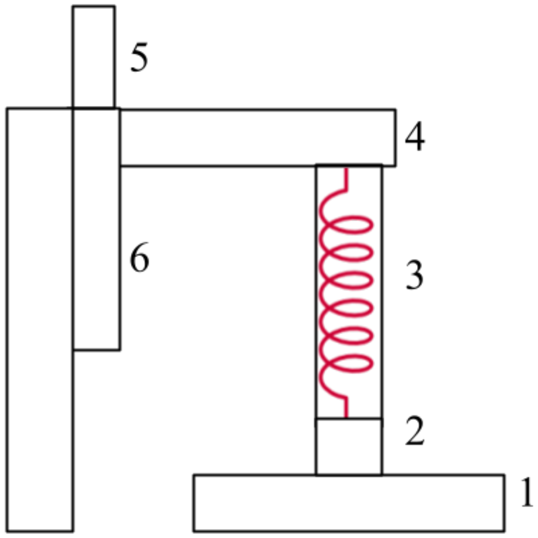


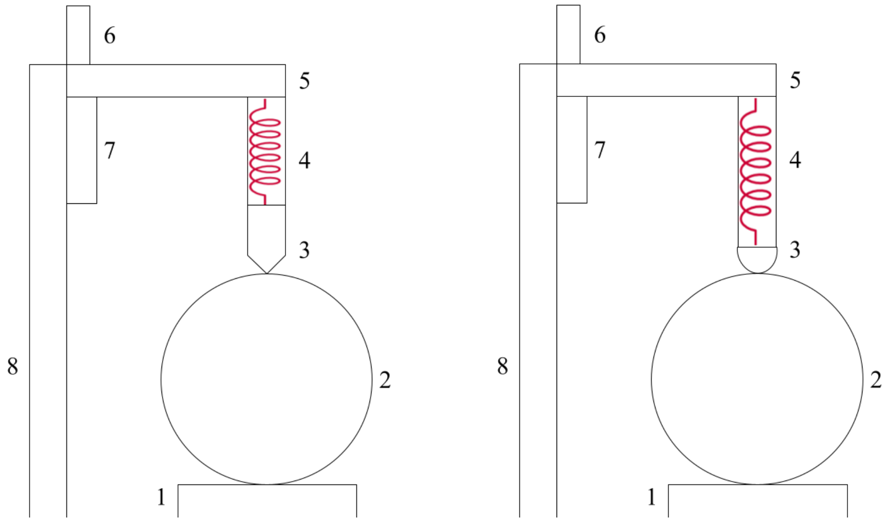
Figure 3: The setup where 1 represent a scale, 2 a cubic tip, 3 a tube containing a spring, 4 a rod attached to a platform, 5 a arm with a millimeter scale that pushes the front of the platform down when turned, 6 a platform.

the movements up to 0.01 millimeters precision.

2.2 Measurement of the Spring Constants of Balloons

The previous setup discussed was also used in order to measure the spring constant of the balloon. However, this time, a balloon filled with 1 atm nitrogen was put on top of the scale, below the tube with a spring and a sharp tip, see Figure 4a.

When the arm with the millimeter scale was turned, the platform with the rod attached to it moved down, causing the spring with the tip to push against the balloon. The rod was pushed down 0.5 millimeters at a time, and the mass was noted. In total, the rod was pushed down 26 millimeters. The experiment was repeated three times and was later tested on a balloon with a pressure of 0.5 atm, and finally one with 0.25 atm.



(a) Sharp tip (b) Dull tip

Figure 4: The setup where 1 represent a scale, 2 a balloon, 3a a sharp tip, 3b a dull tip, 4 a spring inside of a tube, 5 a rod, 6 an arm with a millimeter scale that pushes the platform down when turned, 7 a platform.

3 Results

The spring constant used in the method was found to be approximately 3100 Nm^{-1}. This value was given by the slope of a fitted regression line, see Figure 6 in Appendix.

The values of the slopes showed in Table 1, row 3 are all approximately 1, which indicates that the relation between the forces applied on the balloons and the displacements of the balloons are proportional, according to equation 5. Furthermore, Table 1 and Table 2 show R^2-values near to 1, which means that the regression predictions almost fit the data perfectly.

Table 1: Overview of the slopes of the fitted regression lines on the log-log-plot, and the R^2-values of the regressions.

Measurement	Balloon of evaluation	Slope of log-log-plot line	R^2-value of log-log-plot regression
1	1 atm - dull tip	1.08	1.00
2	1 atm - sharp tip	1.11	0.982
3	0.5 atm - dull tip	1.13	0.992
4	0.5 atm - sharp tip	1.08	0.970
5	0.25 atm - dull tip	1.09	0.995
6	0.25 atm - sharp tip	1.09	0.933

The values of the slopes of the fitted regression lines on a force-indentation curve can be seen in Table 2, i.e. the spring constants of the belloons. The graph can also be observed in Figure 7 in Appendix.

Table 2: Overview of the slopes of the fitted regression lines on the force-displacement-plot, and the R^2-values of the regressions.

Measurement	Kind of balloon	Slope of linear plot line [N/m]	R^2-value of linear plot regression
1	1 atm - dull tip	174	0.998
2	1 atm - sharp tip	157	0.997
3	0.5 atm - dull tip	159	0.998
4	0.5 atm - sharp tip	153	0.997
5	0.25 atm - dull tip	165	0.998
6	0.25 atm - sharp tip	139	0.996

4 Discussion

As previously mentioned, the Hertz model states that the force for a parabolic indenter is proportional to the indentation to the power of 3/2. However, by studying the result, it is clear that for balloons, the force is proportional to the indentation to the power of 1, i.e. the relation between the force and the indentation is linear.

Furthermore, by studying the result, the spring constants seem to be lower when the balloons are poked with sharper tips. This may be due to the fact that the balloons get poked by a smaller area, and that the force applied onto the balloon get lesser. The values of the spring constants may also vary depending on where on the surface the balloons are poked. However, the variation should not be big, and should not have affected the experiment in any bigger extent. Even though these things probably did not affect the result, the things discussed should be taken in account if the experiment was to be repeated.

Why the Hertz model was not applicable to balloons might have several different explanations. As mentioned before, Hertz contact stress refers to local stress that develops when two surfaces come in contact with each other. However, when the balloon is poked with the tip, the pressure is not local since the changes of the balloon are communicated all over its surface and its inside. In other words, the balloon has a uniform pressure.

4.1 Further Studies

For further research, other materials could be used in order to mimic the exosomes at a bigger scale and evaluate their suitability. Optimally, a spherical material could be used since exosomes are spherical. A material that perhaps could be examined in further research is hydrogel, it is both isotropic, like exosomes, and doesn't contain gas. Then, instead of only measuring the spring constants of different hydrogels, the spring constants could be used in order to calculate their bending modulus. Thereafter, if the Hertz model is applicable to hydrogels, Young's modulus could be calculated. Subsequently, the two moduli, the bending modulus and Young's modulus, could together provide an estimate model of its elasticity.

For further research, other methods of measuring the elasticity of exosomes could also be examined, since the Hertz model has flaws.

5 Conclusion

The study showed that balloons are not suitable models for exosomes, since the Hertz model are not applicable to them. However, in further research, other materials and methods could be examined.

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A Appendix

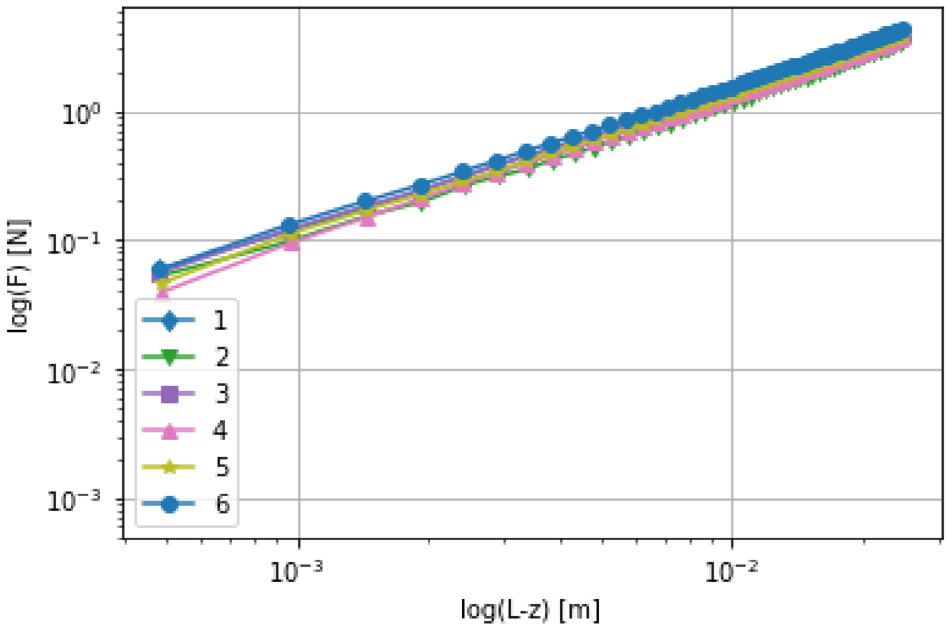

Figure 5: A graph showing $\log_{10}(F)$ plotted against $\log_{10}(L-z)$, with fitted regression lines. The different numbers on the left stand for the different experiments done, where 1 is the experiment with the balloon with 0.25 atm intended with a dull tip, 2 the balloon with 0.25 atm intended with a sharp tip, 3 the balloon with 0.5 atm intended with a dull tip, 4 the balloon with 0.5 atm intended with a sharp tip, 5 the balloon with 1 atm intended with a sharp tip, 6 the balloon with 1 atm intended with dull tip.

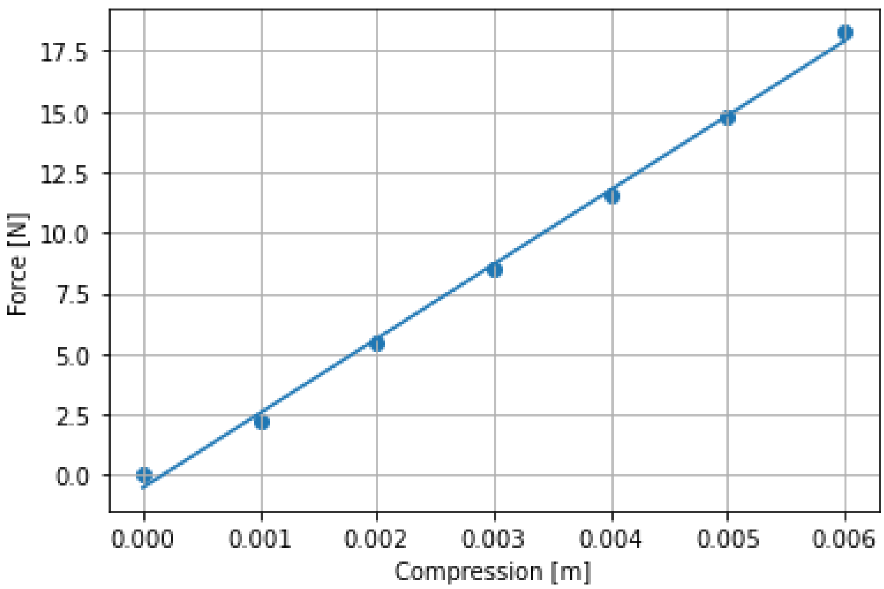

Figure 6: A graph where the y-axis represents the force acting upon a spring, and the x-axis the compression of a spring.

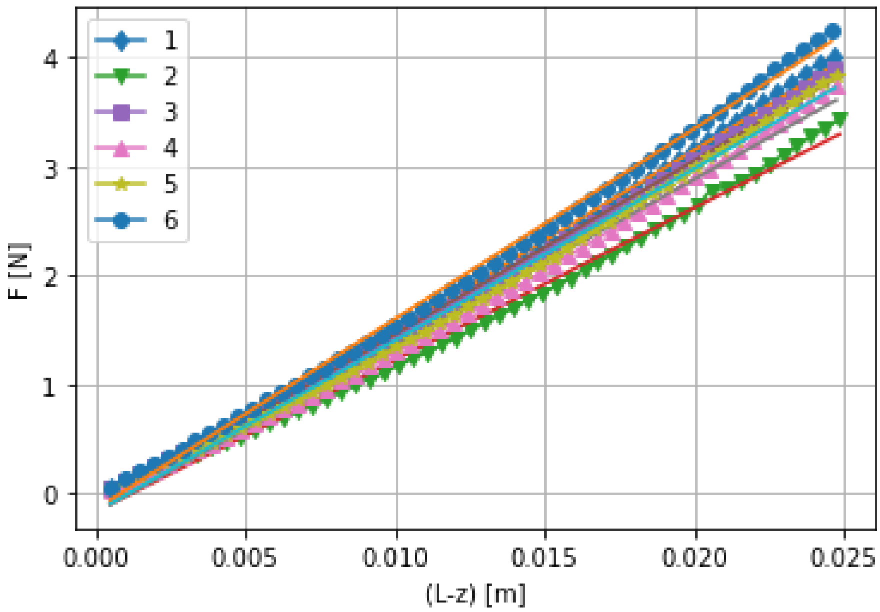

Figure 7: A force-indentation graph of balloons with fitted regression lines. The different numbers on the left stand for the different experiments done, where 1 is the experiment with the balloon with 0.25 atm intended with a dull tip, 2 the balloon with 0.25 atm intended with a sharp tip, 3 the balloon with 0.5 atm intended with a dull tip, 4 the balloon with 0.5 atm intended with a sharp tip, 5 the balloon with 1 atm intended with a sharp tip, 6 the balloon with 1 atm intended with dull tip.