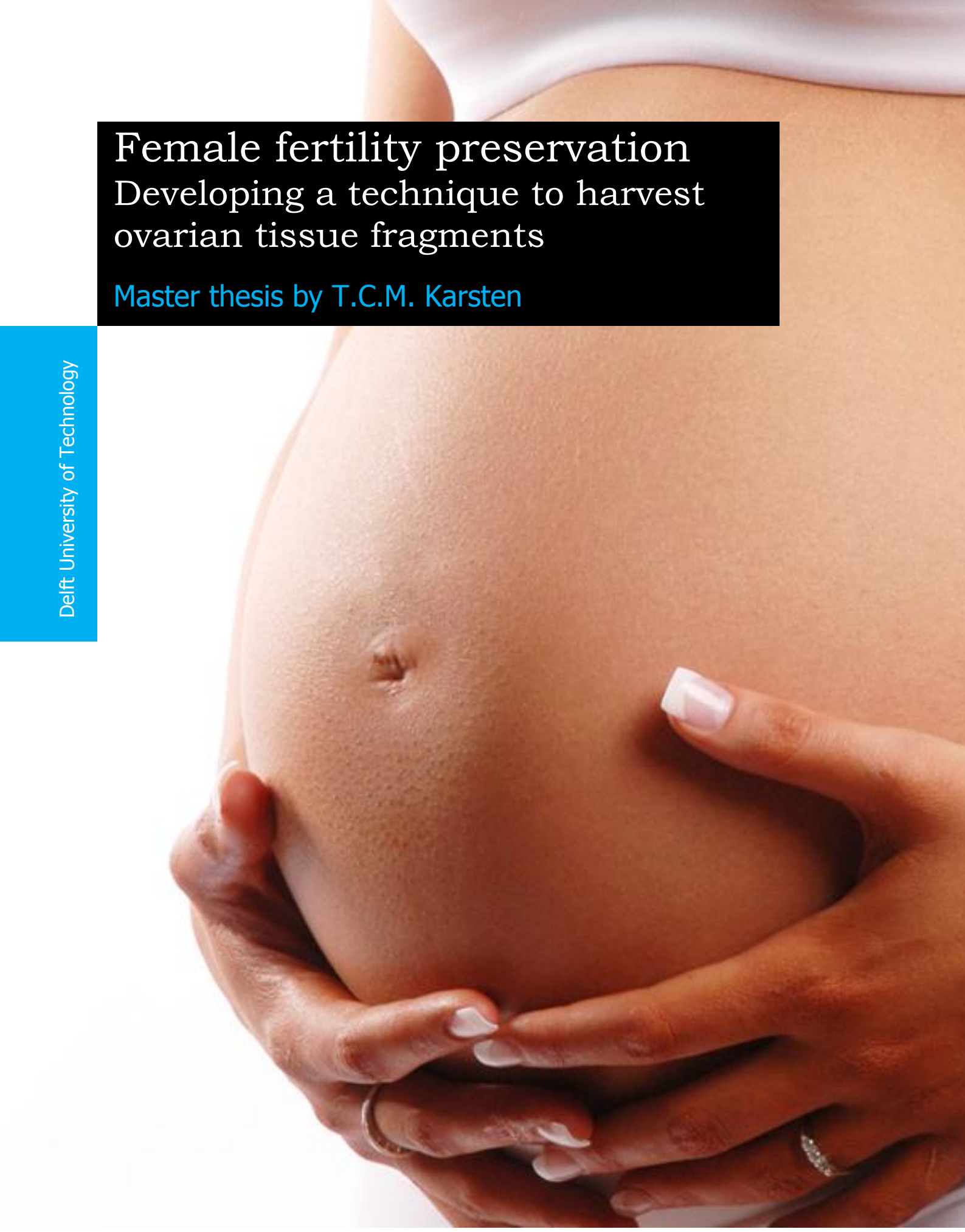
Female fertility preservation

Developing a technique to harvest ovarian tissue fragments

Master thesis by T.C.M. Karsten

Delft University of Technology



TU Delft Delft University of Technology

Challenge the future

Female fertility preservation

Developing a technique to harvest ovarian tissue fragments

By

T.C.M. Karsten

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Preface

This Master thesis is the final product of my Master Biomedical Engineering. I specialized in the subject Medical Instruments and Medical safety. I came into contact with this subject during a course called Medical Device Prototyping. The subject female fertility preservation caught my interest immediately and I am glad I got the opportunity to create my graduation thesis around this subject. I want to thank J. J. van den Dobbelsteen for his supervision during this study, Filip Jelinek for advice during the start, Frank Willem Jansen for giving me the opportunity to work on such an interesting subject, Ben van der Meer for keeping me structured, Tonke de Jong and Maaike de Jong for moral support and the enjoyable lunch and coffee breaks.

T.C.M. Karsten
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Abstract

Young women who suffer from cancer have a high change of becoming infertile as a consequence of the treatment. They would benefit from an effective fertility preservation method that allows natural conception. A new method, called autotransplantation of ovarian tissue, has much potential but improvements can be made. The method entails that ovarian tissue is harvested before treatment and frozen until future use. When the patient is in remission the tissue is transplanted back into the patient, which will allow the patient to become pregnant in a natural way. In order to improve the success rate and facilitate further research, a new technique for harvesting ovarian fragments was researched. The goal of this study was to develop a technique to harvest ovarian tissue fragments with controlled dimensions in a minimally invasive way. The technique had to satisfy two functions, namely holding the ovary and removing a tissue fragment. A concept technique was developed where suction was used to hold the ovary and a knife was used to cut a tissue fragment with an impulse. An experimental set-up was developed to test this concept technique. The use of the set-up with chicken filet showed a proof of principle. The efficacy of the concept technique was proven, as well as the accuracy and precision of the length of the obtained fragments. However, the set-up was not effective in producing fragments origination from ovarian tissue. Suggestions for future research include the use of active suction to hold the ovary and a reciprocating knife to cut a tissue fragment.

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Contents

1 Introduction

Young women who are diagnosed with cancer often lose their fertility due to the treatment they receive. A new method to preserve their fertility is to harvest ovarian tissue before treatment and freeze it until they are cured. Transplanting this tissue back into the patient allows the patient to become pregnant in a natural way. Although this is a promising method, it is not optimal yet. In order to facilitate further research and improve the success rate of this method a new technique for harvesting ovarian tissue is researched in this study. In the following sections the background of the method and the benefits of a new technique are described.

1.1 Cancer survivors

Around 2300 women below the age of forty are diagnosed with cancer each year in the Netherlands alone [1]. These are women in their fertile age. Fortunately more and more people survive cancer due to improvements in diagnostics and treatment. At this moment the survival rate for patients between the age of 18 and 44 is 75%, ten years after diagnosis [2]. However, treatment is not optimal yet. There are many downsides to chemotherapy and radiation because these methods do not only attack cancer cells, but all the cells in the patient's body [3].

1.2 Quality of life

Since more and more people survive cancer, the question rises how we can improve the quality of life for these survivors. One of the major issues for girls and women is the loss of ovarian function due to treatment. The ovaries play an important role in the endocrine (hormonal) system as they are one of the locations where hormones are produced. The situation in which women reside when their ovaries are dysfunctional is similar to the postmenopausal state, which has many drawbacks. In postmenopausal women bone density is negatively influenced and can result in osteoporosis. It is also believed that menopause is a risk factor for severe neurological disorders, such as Alzheimer's disease [4]. To diminish the negative effects of ovarian failure hormone replacement therapy is offered. However, the biggest consequence of ovarian failure is infertility and this cannot be counteracted with hormone replacement therapy. The gift of conceiving a child is something every women should be able to experience. Therefore fertility preservation is one of the pillars in improving quality of life for cancer survivors. Although techniques for fertility preservation are developing rapidly, doctors do not always discuss fertility preservation options with their patients. A research amongst pediatric oncologists in the UK from 2004 showed that the risk of infertility was discussed in 62% of the cases, while only 8% were offered fertility preservation options [5]. These results show that information about fertility preservation options should be spread amongst doctors in order to give young girls the chance of becoming a mother

after surviving cancer. It also shows that the number of patients that will request fertility preservation in the future will rise. This creates opportunities to research the area of fertility preservation more.

1.3 Female fertility preservation

A broad range of methods to preserve fertility currently exist. In this study, female fertility is defined as the ability of a women to give birth to a child that is genetically tied to the mother. This definition excludes options like egg cell donation and adoption. The most established method to achieve preservation of female fertility is the cold storage of embryos (embryo cryopreservation). The process starts by stimulating the ovaries with hormones in order to start further development of multiple egg cells (oocytes). This development requires 2 to 6 weeks depending on where in the menstrual cycle the patient is [6]. Before cancer treatment can begin the mature oocytes are harvested and, after fertilization, frozen and stored in liquid nitrogen for future use [7]. Because cancer treatment is delayed until the oocytes are developed, this method is not suited for women who need immediate treatment. Furthermore, since this method requires sperm for fertilization it is not optimal for girls or women without a male partner and not suitable for girls or women that have ethical, moral or religious issues with sperm donation. To circumvent the delay in treatment and the need for sperm several other methods are being researched today. Apart from cells being frozen to preserve their function, it is also possible to preserve complete tissues by freezing them. The advantage of freezing ovarian tissue is that no hormonal stimulation is required. Although a surgery is necessary to harvest the tissue, it will not delay cancer treatment [8]. Prepubertal girls can benefit from this method, as well as women with estrogen-sensitive cancers, and women with highly aggressive malignancies where rapid initiation of treatment is required [7, 9]. A method that uses the technique of preserving tissue is called ‘autotransplantation of cryopreserved ovarian tissue’.

1.4 Autotransplantation of cryopreserved ovarian tissue

The upcoming fertility preservation method ‘autotransplantation of ovarian tissue’ is most interesting because natural conception is made possible again. A second advantage is that hormone replacement therapy will not be necessary as long as the transplanted fragments are viable. This method entails that ovarian tissue fragments (cancer free) are harvested before treatment and frozen for future use. Upon use, the tissue fragments are thawed and transplanted back into the same patient (autotransplantation), preferably in the ovaries. The fragments should then regain function and restore the endocrine function of the ovaries as well as restore fertility for as long as the fragments are viable. Although the method is quite new, the interest in ovarian transplantation started already in the 18th century but the first attempts to transplant tissue from one human to another were realized in the 1900s. Immune reactions of the receiving patient prohibited a positive outcome. These immune reactions would not occur when tissue from the same patient could be used. However, autotransplantations were not possible yet due to a lack of preservation methods for the ovarian tissue. In 1948 the first cryoprotectant, glycerol, was discovered. Glycerol prevents the formation of ice crystals during the freezing process that would otherwise cause damage to the cells. This discovery led to the pioneering work of transplanting autologous cryopreserved tissue in the 1950s. Realization of its clinical importance came in 1990 and after good results in animal models. By today already 24 healthy human babies have been born as a result of autotransplanted cryopreserved ovarian tissue [8, 9].

1.5 Objective

The objective of this study:

To improve the method of autotransplantation by developing a technique to harvest ovarian tissue fragments with controlled dimensions in a minimally invasive way.

First an explanation on minimally invasive techniques. Minimally invasive surgeries are also known as keyhole surgeries or laparoscopies. Although an open procedure is easier to perform by a surgeon, a laparoscopic procedure is typically preferred as there are many benefits for the patient: the chance of infections is lower, and the patient recovery time is shorter. Esthetically speaking, a laparoscopy leaves less scar tissue, which is of great interest to many patients [10]. Besides the advantages of a minimally invasive procedure, the technique proposed in the objective will have multiple advantages over the current harvesting procedure, namely:

- Improving the lifespan of the fragments due to controlled fragment size
- Increase in transplantation area due to both ovaries remaining in situ;
- A decrease in time and difficulty level of the post-operative steps of the harvesting procedure;
- A step towards standardization of the method due to the controlled fragment size, which will facilitate further research into the other parameters of this fertility preservation method.

In the following sections these advantages will be explained and the ideal fragment size will be discussed.

1.5.1 Lifespan

The lifespan of the transplanted fragments is expected to be around 5 years and is mostly determined by the number of oocytes present in the fragments. Oocytes are not distributed equally throughout the ovary. An ovary can be subdivided into different layers (Figure 1): the single cell layer that covers the ovary is called the germinal epithelium and consists of epithelial cells. Below this layer lies a dense connective tissue layer called the tunica albuginea. The interior tissues are the superficial cortex that contains the oocytes and the deeper medulla that contains the blood vessels [11]. Most oocytes are present at a distance of 0.8mm from the surface [9]. For this reason fragments should at least include the cortex of the ovary. However, there is a limiting factor for the thickness of the fragments. The number of oocytes in the fragments is affected by several factors: oocyte density before cryopreservation (age dependent); chemotherapy before harvesting of ovarian tissue; fragment size; freezing and thawing process; and the potential of the transplantation site to form new blood vessels necessary for vascularization of the fragments [9]. The delay in vascularization of the fragment is considered to be a serious problem of transplanting ovarian tissue and it is estimated that more than 50% of the oocytes are lost after

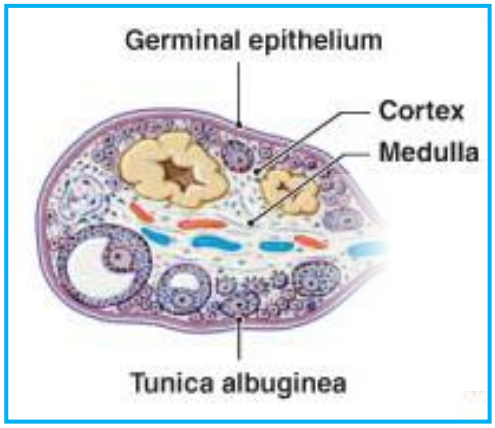


Figure 1: a cross section of the ovary. The ovary can be subdivided into different layers: germinal epithelium, the tunica albuginea, the superficial cortex that contains the oocytes (egg cells) and the deeper medulla that contains the blood vessels [11]

reimplantation due to ischemia (lack of blood supply). Reoxygenation of the implanted fragments occurs from the 5th day onwards, which means that the first 5 days the fragment is without blood supply which causes the decrease in oocytes [12, 13, 14, 15]. In a study, where fresh cortical tissue slices were transplanted between monozygotic twins, Silber et al. [16] state: "it was felt important to prepare a cortical tissue slice no thicker than ~1.0mm to facilitate rapid vascularization". A thick tissue slice is more difficult to vascularize. Therefore it can be concluded that thin slices are preferred. At the moment no harvesting procedure exists that results in tissue fragments with controlled dimensions. The technique suggested in the objective would therefore help to improve the lifespan of the fragments by making fragments with controlled dimensions.

1.5.2 Transplantation area

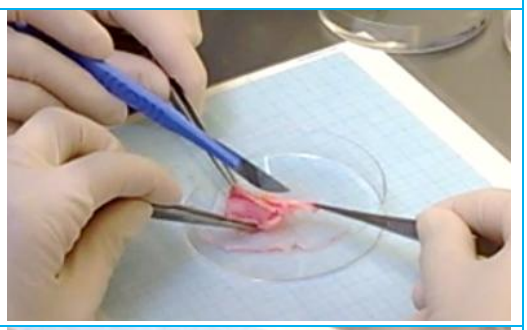
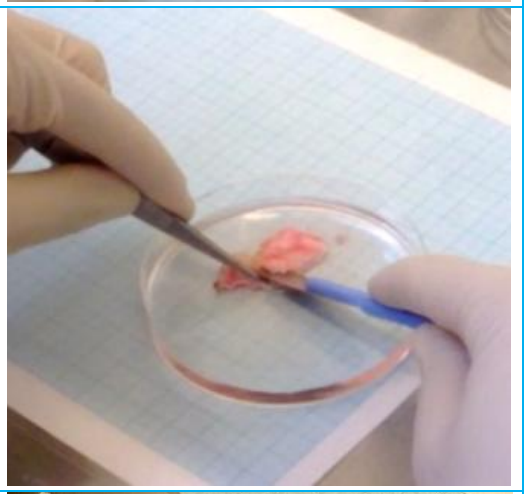
If the patient is in complete remission and desires pregnancy, the ovarian tissue fragments are thawed and prepared for transplantation. Not all harvested fragments are transplanted back in a single procedure. A part stays frozen and can be used for a second or third transplantation procedure if the first is not successful or if the lifespan of the transplanted fragments has ended. An ovary will provide the most optimal environment for oocyte development, obviously, because it is the natural location for oocytes. Since fast vascularization is important for oocyte survival the best site will be under the cortex, close to the medulla. As described before, the medulla contains the veins and arteries of the ovary. To gain access to the medulla the ovary can either be decorticated or an incision can be made in the cortex. The tissue slices are then either placed on the exposed medulla or in the incision [9]. If both ovaries remain in situ, both ovaries can be used as transplantation site and the area for transplantation will be increased as opposed to the current procedure. The technique proposed in the objective will leave both ovaries in situ and will therefore increase the transplantation area.

1.5.3 Harvesting procedure

The existing harvesting procedure is performed minimally invasively. For the minimally invasive harvesting procedure, there are two general approaches: The surgeon can remove one whole ovary (oophorectomy), separate the cortex and freeze the tissue, or the surgeon can take cortical biopsies during the procedure which are ready for cryopreservation [17]. In Table 1, the post-operative steps of the current harvesting procedure that is used in the Leiden University Medical Center is described. First a minimally invasive oophorectomy is performed. The removed ovary is send to the laboratory after which the described steps take place. It can be seen that the post-operative steps are quite cumbersome and take up much time. Two technicians are needed to obtain the tissue fragments while there is no control over the fragment dimensions.

Taking biopsies has an advantage that tissue samples can be taken from both ovaries, while they remain in situ and can be used as transplantation site. The advantage of removing a whole ovary is that the procedure is easy to perform and the complete cortex can be divided into slices and frozen. At the moment taking several biopsies is a more difficult procedure. The technique proposed in the objective aims to make the procedure of taking biopsies much easier to perform. Additionally, there will be no need for timely post-operative steps because the fragments that result from the operating room will be ready for cryopreservation.

Table 1: Current harvesting procedure of the Leiden University Medical Center

The Leiden University Medical Center (LUMC) has their own method to obtain the ovarian cortex. In the operation room (OR) one ovary is removed from the patient (unilateral oophorectomy). This ovary is send to the lab immediately where two lab technicians tend to the decortication of the ovary. During the decortication of the ovary, several scalpels were needed, because the stiffness of the tissue created blunt scalpels quickly. It was estimated that around three scalpels were needed for one complete ovary. * Thanks to L.A.J. van der Westerlaken from the LUMC		
1	First the ovary is cut in two halves in a petri dish. One technician holds the ovary with two pair of tweezers, while the second one holds one pair of tweezers and one scalpel. The scalpel makes cuts with a sliding movement from up to down the center line of the ovary until the ovary is in two halves.	
2	Then, each lab technician takes one half of the ovary in a separate petri dish and starts cutting out the medullar from the inside of the ovary. Cutting is done with one pair of tweezers and one scalpel. The pair of tweezers holds the ovary in place, while the lab technician scrapes with the scalpel. The cutting movement of the scalpel starts at the point that is hold by the tweezers and moves outwards. The position of the blade starts about perpendicular with respect to the table and ends almost parallel to the table. In this way the medulla is cut out of the ovary layer by layer until only the cortex is left. The medulla has many blood vessels and has therefore a pinkish color. The color of the cortex is white. This makes it easy for the technicians to see when there is no medulla left.	
3	Subsequently, the cortex is cut into fragments. Again a pair of tweezers is used to hold the cortex, while cutting is done by pushing the scalpel down and making small rolling movements back and forth that follow the curve of the blade. The sizing of the fragments is based on millimeter paper that can be seen through the petri dish. The target dimensions of the fragments are 5x(5-15)mm. The final fragments are of various sizes. On average one ovary results into twenty fragments.	
4	In the final steps the fragments are first put into a tube with a cryoprotective agent (CPA). After incubation on a tube roller/mixer the fragments are transferred into an ampoule of 1.5mL and frozen according to the cryopreservation protocol.	

1.5.4 Standardization

Even though the method of autotransplantation of ovarian tissue fragments has proven itself, the importance of many factors (transplantation site, fragment size, fragment attachment, etc.) is still unknown. Therefore doctors choose specific parameters based on own judgment and not based on known pros and cons. By standardizing the procedures, research could be done to the effect of changing certain factors. In this study, the focus lies on the harvesting procedure, because it is logical to start standardizing at the beginning. A standardized harvesting procedure could lead to a standardized transplantation procedure. Once the optimal fragment size is known, a technique for transplanting these controlled fragments will be easier to develop. The technique proposed in the objective will aim to result in fragments with controlled dimensions and will therefore be a good start to standardize all the procedures of the autotransplantation method.

1.5.5 Fragment size

Fragment size is important for the success rate of the transplantation procedure, but it is also important for the cryopreservation procedure. The harvested tissue needs to be stored for a long time during which tissue function cannot be affected. In order to suspend the metabolic processes in the ovarian tissue the slices are cooled to -196°C using liquid nitrogen. Freezing tissue is not as simple as it sounds. Just immersing the tissue in liquid nitrogen will damage the cells because the water in the cells will form crystals. The crystals take up a greater volume than water and this will damage the cell structure [18]. In order to prevent this cryoinjury, several methods exist that use cryoprotective agents (CPAs) to safely freeze human tissue. CPAs prevent ice formation in cells, but can also be toxic when concentrations become too high. To allow penetration of the CPAs into the inner parts of the tissue, an equilibrium period is used during the procedure. In an experiment by Silber et al. [19], the number of surviving oocytes in ovarian tissue after cryopreservation were compared to fresh ovarian tissue. The survival rate of oocytes in fresh tissue was around 89%, while cryopreserved tissue resulted in a surviving rate of only 41.7%. The CPAs that are used need to perfuse into the tissue. Too large fragments will be difficult to perfuse. Therefore there is a limit to the size of the fragments, which is 2x4x12mm for the current freezing technique [18].

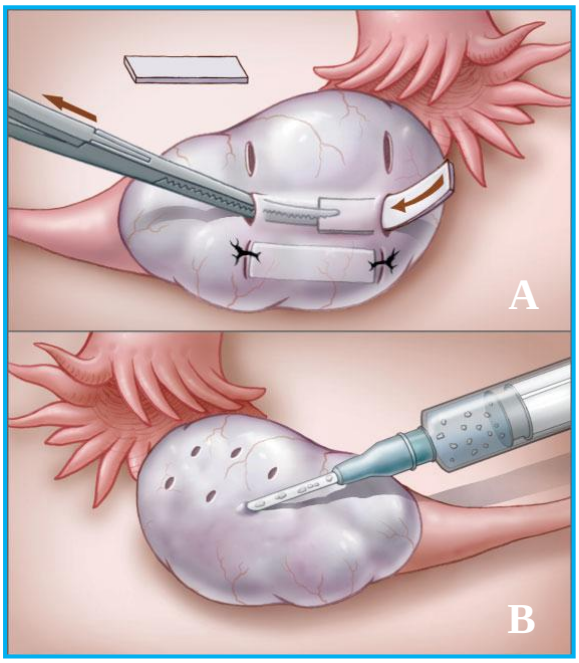


Figure 2: Comparison of two transplantation techniques. (A) Large strips of 8-10 x 5mm were placed under de cortex of the left ovary. (B) Tiny ovarian fragments were injected into the right ovary. Only the left ovary, containing the large strips, regained function [21].

The ideal size for a successful transplantation procedure is harder to determine. Most clinicians agree on the thickness of the ovarian cortical slices, however, there is no consensus yet about the other dimensions of the slice. A review of orthotopic autotransplantation cases from three centers showed no difference for different fragment sizes with respect to restoring ovarian endocrine function. Either large strips of (8-10)x5mm or small cubes of 2mm3 were used [9]. Donnez et al. [20], however, advise the use of cortical strips since it is easier to attach them to the transplant site. Meirow et al. [21] tried two

techniques; one placing strips of 15x5mm under the cortex of the left ovary; and the second injecting very small fragments (size unknown) into the right ovary of the same patient (Figure 2). The strips were successful in restoring ovarian function, in contrast to the small fragments, which did not regain function. Although the exact size of the small fragments used by Meirow et al. is not mentioned, the results do indicate that fragment size is important. In addition, more cuts are needed to obtain smaller fragments and every cut potentially damages an oocyte. Therefore, one could argue that the bigger the size of the fragment, the better. Of the 24 births due to autotransplantation, 17 were found to be described in articles. They were studied and the results are presented in Table 2. Although there is no real consensus about fragment size, the data that is obtained so far shows that successful fertility preservation can be reached by using fragments of no more than 2mm thick and between 2-15mm wide and long. However, most oocytes lie in the outer 0.8mm of the cortex and vascularization is improved with thinner slices. Therefore the targeted fragment thickness for this study will be 1mm.

Table 2: Successful autotransplantation procedures and the used fragment size. (Age is the age of the patient at the time of cryopreservation, pc = pieces, Spont = spontaneous pregnancy, IVF = In vitro fertilization).

Age	Fragment size	Live birth		Reference
		Spont.	IVF	
25	1pc (12x4x1mm) + 67pc (2x2x1mm)	+		Donnez et al. [12]
18	6pc (7-8x4x1-2mm)		+	Donnez et al. [22]
17	2x2x2mm	+		Donnez et al. [23]
28	3pc(15x5x1-2mm)		+	Meirow et al. [21]
24	2pc (5x5x2mm)	+		Demeestere et al. [24]
26	10pc (5x5x1-2mm)		+	Andersen et al. [25]
27	6pc (5x5x1-2mm)	+ (2nd)	+ (1st)	Andersen et al. [25, 26]
24	N.A., <1mm thick	++		Silber et al. [19]
25	N.A., <1mm thick	+		Silber et al. [16]
20	3pc (5x10mm)	+		Roux et al. [13]
36	N.A.		++ (twins)	Sanchez et al. [27]
19	15-20x2-4x0.35mm		+	Revel et al. [28]
25	6pc (1x2x1mm)	+		Dittrich et al. [29]
21	17pc (5x5x1mm)	+		Revelli et al. [14]

1.6 Requirements

The goal of this study is to develop a technique to harvest ovarian cortical fragments with controlled dimensions in a minimally invasive way. This method has to satisfy the following requirements:

- Tissue fragment dimensions should be no more than 1mm thick and have a fixed size between 2-15mm wide and long.
- The method should be applicable for minimally invasive procedures, preferably for a trocar size of 5mm.
- The method should not damage the cortical tissue fragments or the patient
- The method should be save to use for surgeons

1.7 Reading guide

In the following chapters the steps will be described that were taken in order to develop a new technique to harvest ovarian tissue. In chapter 2, the development of the concept technique will be described as well as the design details of the set-up. Additionally, the experimental methods that were used to test the concept technique are also described in chapter 2. Chapter 3 will show the results from these experiments. The outcome will be discussed in chapter 4 and finally, a conclusion will be given in chapter 5.

2 Materials & Methods

2.1 Concept generation

The goal of this study is to find an instrument to harvest ovarian tissue with controlled dimensions in a minimally invasive way. Besides the set requirements, the instrument has to fulfill two functions, namely holding the ovary and removing a tissue fragment. A method is already developed that results in precise defined dimensioned fragments; the tissue slicer [30]. However, the tissue slicer is developed for laboratory use only and not for clinical use. This method cannot be performed minimally invasive. There are, however, existing clinical procedures in which tissue is removed minimally invasive. One can think of polyp removal, tumor removal or taking biopsies. For these procedures special minimally invasive instruments are developed. By analyzing the functioning of these instruments a concept can be formed for the minimally invasive harvesting instrument. First, the method using the tissue slicer will be discussed and subsequently two minimally invasive instruments that are developed for the removal of tissue are presented. Finally, an instrument that is used to stabilize tissue is discussed. All the instruments are summed up in Table 3 together with their fulfillment of the two desired functions (holding tissue, and removing a fragment).

2.1.1 Tissue slicer

Silber et al. [30] describe a method of cutting ovaries to guarantee a fragment thickness of 1mm. This tissue slicer consists of two plates and a knife. The first plate is 1mm thick and has an open space in the middle of 10x10mm. This plate is pushed on the surface of the ovary. A scalpel is used to cut the borders of the open space (Figure 3A). A second (closed) plate is put on top of the first plate. While keeping these plates tightly together, the knife is used to cut between the first plate and the ovary (Figure 3B). The result is a fragment of 10x10x1mm (Figure 3C). This method fulfills exactly the functions

Figure 3: Technique of the tissue slicer. This tissue slicer consists of two plates and a knife. The first plate is 1mm thick and has an open space in the middle of 10x10mm. This plate is pushed on the surface of the ovary. (A) A scalpel is used to cut the borders of the open space. A second (closed) plate is put on top of the first plate. (B) While keeping these plates tightly together, the knife is used to cut between the first plate and the ovary. (C) The result is a fragment of 10x10x1mm [30].

needed for the harvesting instrument. However, this method is developed for a laboratory environment and is not suited for clinical use, let alone minimally invasive. By analyzing the method, a concept for the minimally invasive harvesting tool can be generated. In this method preparation of the ovarian cortex is done by cutting the borders of the desired fragment by using the open space of the first plate as a template. Holding the ovary is achieved by clamping the ovary between the table and the plates. A tissue fragment is removed from the ovary by cutting with a straight blade in a linear fashion. This blade is kept at a distance of 1mm from the surface of the ovary by the thickness of the first plate. The second plate fulfills the function of keeping the tissue from bulging out of the first plate. Finally, the knife cuts a fragment with precise dimensions (width, length and depth).

2.1.2 Core biopsy instrument

A biopsy is taken in order for the tissue sample to be analyzed in a laboratory. This procedure is mostly done to determine if the patient has cancer [31]. A commonly used tool for taking breast biopsies is a core biopsy instrument [32, 33]. The part of the core biopsy instrument that interacts with the tissue consists of a hollow cutting cannula with a sharp distal end and a solid stylet that includes a transverse slot/specimen notch adjacent to a sharp distal end (Figure 4). The cannula slides directly over the stylet. Upon use, the stylet is plunged into the tissue that needs to be biopsied. When it is in place, the cannula is shot over the stylet leaving a tissue sample within the transverse slot of the stylet. Usually a spring mechanism is used to shoot the cannula over the stylet [34, 35, 36]. One of the drawbacks of such a device is that is does not always result in a sample of a sufficient size [34]. To overcome this problem, further developments of this instrument include the addition of a vacuum force to a hollow stylet in order to draw the tissue into the transverse slot before shooting the cannula. The vacuum can be applied with use of a standard syringe [34]. The two desired functions are fulfilled in the following way: to hold the tissue the core biopsy instrument sometimes makes use of suction that is applied with use of a syringe. Removal of a tissue fragment is done with a knife that is shot over the tissue by an impulse.

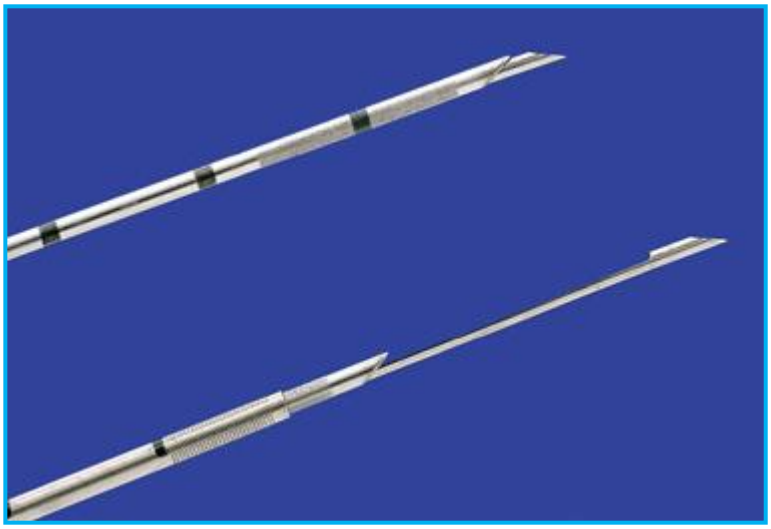


Figure 4: Tip of the core biopsy instrument. It shows the solid stylet with a transverse slot that holds the tissue sample and the hollow cutting cannula that slides over the stylet [33].

2.1.3 Hysteroscopic tissue removal device

The hysteroscopic tissue removal device is developed as a minimally invasive tool for the removal of 3cm uterine fibroids and 5cm uterine polyps in 10 minutes or less. Uterine fibroids are noncancerous growths of the smooth muscle tissue in the uterus [37]. Just like fibroids, polyps are growths in the uterus. Polyps, however, originate from the inner lining of the uterine

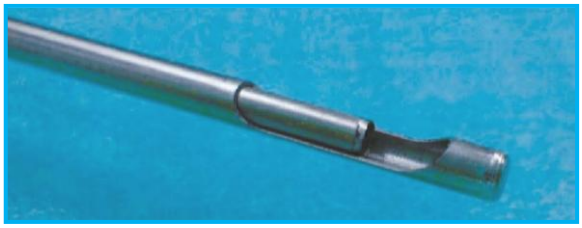


Figure 5: The tip of the hysteroscopic tissue removal device, showing an outer tube with a side-facing cutting window and a tubular cutting blade that can rotate and reciprocate inside the outer tube [39].

wall [38]. The part of the instrument that interacts with the tissue consists of an outer tube with a side-facing cutting window and a tubular cutting blade that can rotate and reciprocate in the outer tube (Figure 5). The tubular cutting blade is also connected to a vacuum source that continuously aspirates resected tissue through the window into the outer tube [39]. Although suction is used in this instrument, it is not used to hold the tissue, but simply to remove loose tissue pieces. As for the two desired functions only the removal of a tissue fragment is satisfied in the manner of a rotating and reciprocating knife.

2.1.4 The octopus tissue stabilizer

The octopus tissue stabilizer is an instrument developed to allow open heart surgery without use of cardiopulmonary bypass (heart-lung machine) (Figure 6). It can stabilize part of the heart while the heart is beating, allowing surgery to take place on the immobilized area. The instrument consists of two parallel paddles that each have three suction cups. Once a suction pressure of 400 mmHg (53 kPa) is reached, the area around the paddles is immobilized [40]. This instrument only satisfies one of the desired functions, namely holding tissue with use of suction.

Figure 6: The octopus tissue stabilizer is used to stabilize part of a beating heart. Two parallel paddles each have three suction cups [40].

2.1.5 Instrument concept

The devices above were used as inspiration for the conceptualization of the harvesting instrument. In Table 3 the techniques are compared in their ways to fulfill the two desired functions, namely removing tissue and holding tissue. The first function of the instrument is to remove a tissue fragment. Both the core biopsy tool and the hysteroscopic tissue removal device use a tubular knife to cut tissue. In the case of harvesting ovarian tissue, the fragments have a restriction to the thickness. The desired shape of the fragments is a flat surface. A tubular knife does not correspond with this requirement. However, as the current harvesting procedure also uses a scalpel for cutting ovarian tissue fragments, the use of a knife to cut tissue seems obvious. For this reason the choice is made to integrate a scalpel into the instrument concept for removing tissue fragments. A scalpel can be moved in several ways to cut tissue. The movement of the knifes in the above described devices also differ. The biopsy tool uses a spring mechanism to create an impulse of the knife in one direction. The hysteroscopic tissue removal device can rotate and reciprocate to make the desired cut. A simple mechanically driven instrument is preferred because it is easier to make, easier to use and easier to market. With increasing movements and directions of the knife the instrument becomes increasingly more complicated. Therefore, the concept for the harvesting instrument makes use of a knife that cuts tissue by impulse.

Table 3: Comparison of inspirational techniques and instrument. (MI = minimally invasive).

Technique	MI	Removing tissue	Holding tissue
Tissue slicer	No	Knife (linear)	Clamped
Core biopsy instrument	Yes	Knife (impulse)	(suction)
Hysteroscopic tissue removal device	Yes	Knife (rotate and reciprocate)	-
Octopus tissue stabilizer	No	-	Suction

The second function the instrument has to fulfill is holding the tissue. The desired ovarian fragments originate from the cortex of the ovary. This is a tough place to cut a fragment. Holding the tissue should prevent the ovary from slipping away when the impulse force of the knife is exerted. The core biopsy tool and the octopus tissue stabilizer described above both make use of a suction force. For the biopsy tool this suction force is used to pull the tissue into the notch. The suction force is created by the use of a syringe. Suction can be used as method to hold the ovarian tissue. This is an effective way to not damage the tissue and it could also help to establish the correct dimensions of the ovarian fragments.

With the two function and their solutions, the concept for the harvesting instrument is as follows: a 5mm hollow rod with a flattened distal end that holds a recessed opening through which the suction could be applied. A knife slides over the flattened part and can cut pieces with a thickness similar to the depth of the recess (Figure 7).

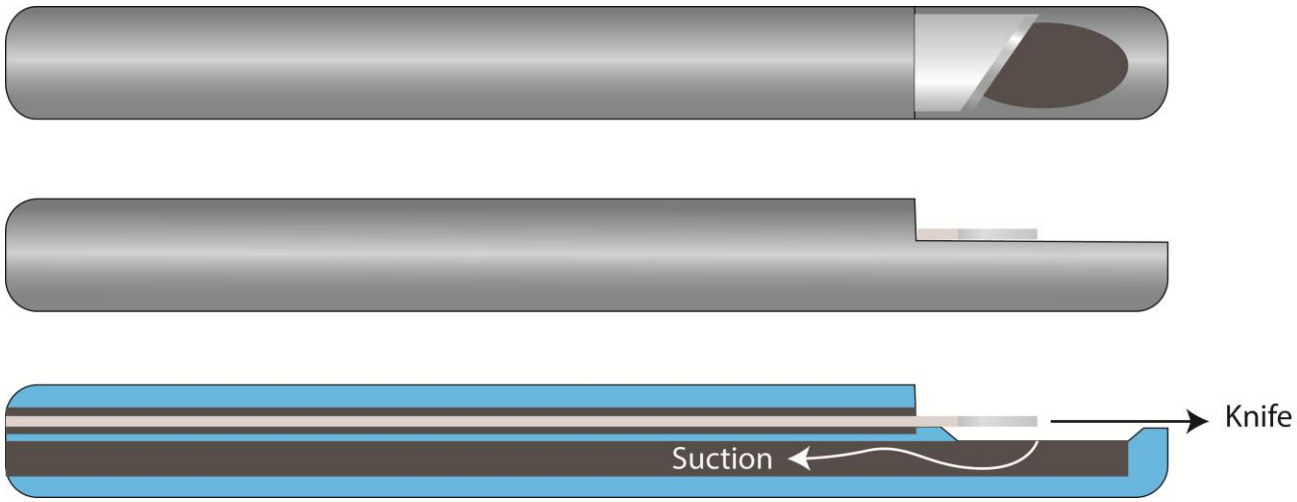


Figure 7: Conceptual design for the harvesting instrument that will result in ovarian fragments with controlled dimensions in a minimally invasive way. The instrument consists of a 5mm hollow rod with a flattened distal end that holds a recessed opening through which the suction could be applied. A knife slides over the flattened part and can cut pieces with a thickness similar to the depth of the recess. (A)Top view. (B) Side view. (C) Cross section of the side view, showing the recess with the suction tube and the knife that slides directly over the recess.

2.2 Description of the functional units

Before developing the concept into an actual instrument, a set-up was made to test the method of using suction to hold the tissue and using an impulse to cut the fragment. For this set-up both functions are designed in separate units, a suction unit and an impulse unit. Creating separate units allows for a change of only one of the functional solutions if the combination does not work as thought. This makes it also possible to find out which function does not work properly. In the following sections the design of both units is described.

2.2.1 The suction unit

The suction unit can be seen as a hollow box with a protrusion on the side in the shape of a Luer-Slip male fitting and a recess of 1mm on top in the shape of an oval with a grid of holes (Figure 8). A syringe can be connected to the protrusion via a tube to apply a suction force. The oval recess functions

as the ‘mouth’ in which the tissue is sucked in to create the final holding of the tissue. The shape and size of the mouth determine the shape and size of the final tissue slice. The initial idea for this experiment was to create several suction units with various mouths. These mouths would have different shapes, sizes and depths. By testing these mouths with various parameters the optimal mouth can be obtained. However, the process in this study did not lead there. In this first instance, the mouth is shaped as an oval of 8x4mm. It seemed favorable to choose a shape without acute angles, e.g. a circle or an oval when using suction. By thinking ahead about the limiting size of the ideal trocar (5mm), the width was determined. In order to create a larger surface area an oval shape was chosen with length twice the size of the width. The depth of the recess determines the thickness of the tissue slice. Since the ideal thickness is 1mm, the depth of the recess was made 1mm. The walls of the recess where finished with a fillet of 0.5mm to the outer shell. To prevent tissue from being sucked into the suction unit, a grid was made in the oval recess. The size and distance between the holes of the grid were determined by the limitations of the production method. In order to obtain an airtight compartment the suction unit was created with a 3D-printer. The suction unit was printed in two parts and then glued together. The printer had an accuracy of 0.3mm, therefore the holes have a diameter of 0.6mm and the center to center distance is 0.9mm, leaving 0.3mm between the holes. The space in the four ‘corners’ of the oval could not fit a regular hole of 0.6mm. Therefore four holes with a diameter of 0.4mm at a center to center distance of 0.8mm from regular holes were added. Again, the space between these holes and the surrounding holes was 0.3mm.

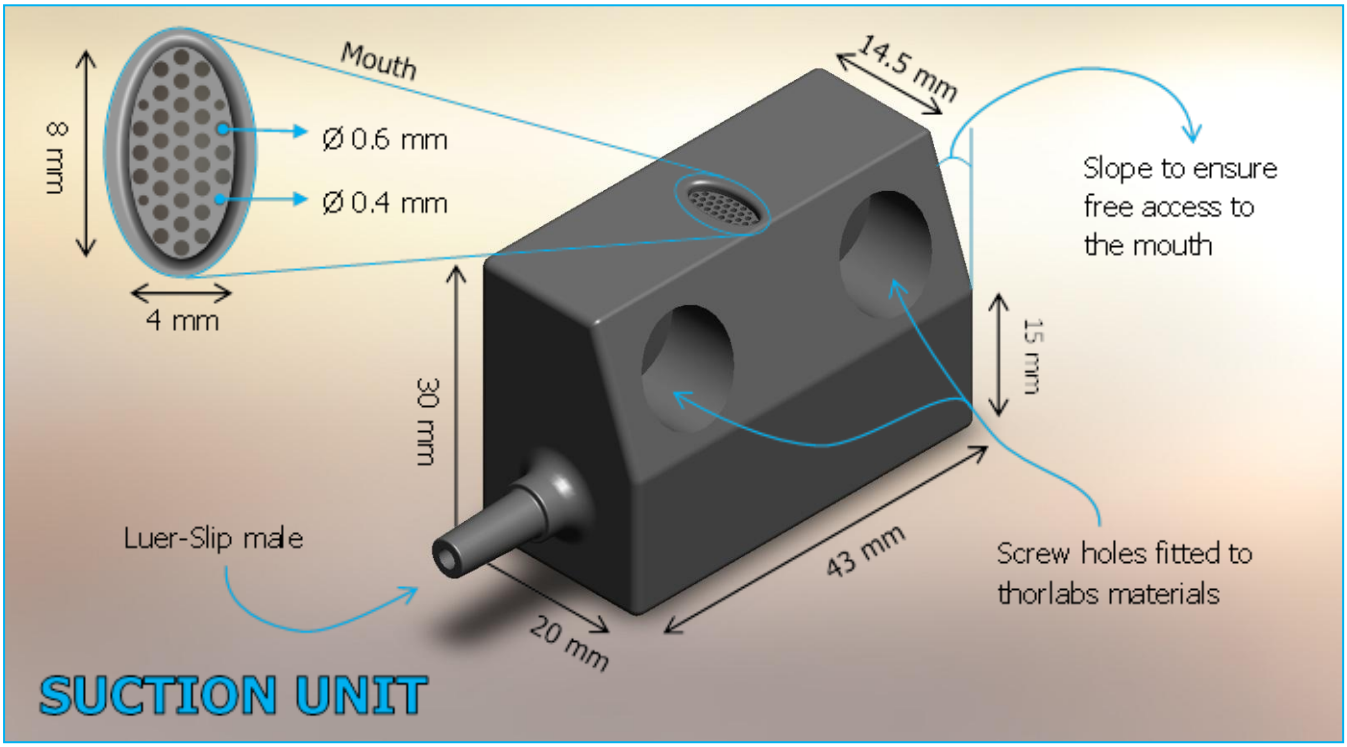


Figure 8: Schematic overview of the suction unit and its important features. In order to obtain an airtight compartment the suction unit was created with a 3D-printer. The suction unit was printed in two parts and then glued together. The suction unit can be seen as a hollow box with 5mm thick walls, a protrusion on the side in the shape of a Luer-Slip male fitting and a recess of 1mm on top in the shape of an oval with a grid of holes. A syringe can be connected to the protrusion via a tube to apply a suction force. The oval recess functions as the ‘mouth’ in which the tissue is sucked in to create the final holding of the tissue. The walls of the recess where finished with a fillet of 0.5mm to the outer shell. To prevent tissue from being sucked into the suction unit, a grid was made in the oval recess.

The size of the total suction unit was partly determined by the distance and size of the two screw holes. These were chosen in order to fit the suction unit to Thorlabs materials. The center to center distance between the holes was 25mm and the hole for the head of the M6 screw had a diameter of 11mm. The width of the suction unit was 43mm, the height 30mm and the depth 20mm. The suction unit was made hollow in order to let air flow from the mouth to the Luer protrusion. The chosen wall thickness of 5mm is over dimensioned but it will ensure the unit can sustain the pressure difference. From the border of the mouth a slope connects the top to the side of the unit. This slope ensures free access to the mouth from that side, while keeping the mouth above the hollow compartment that is inside the suction unit.

2.2.2 Impulse unit

The idea of the impulse unit is that a sharp knife can be shot in a horizontal direction with great force. In order to comply with this idea, the impulse unit is built around a compression spring mechanism (Figure 9). The main rod assembly, that consists of a scalpel holder, a center disk, a spring holder and a handle, slides through two guides. A spring fits around the spring holder and is contained between the center disk and one of the two guides. When the handle is pulled in an outward direction the spring is compressed and stores potential energy. The handle can rotate and get caught by the base of the unit, hereby locking the spring in its compressed position. When the handle is rotated back to the free position, the energy is released and the spring pushes the rod in the opposite direction of pulling and an impulse is created. See appendix IV for technical drawings of the components of the impulse unit.

The part of the rod assembly that holds the scalpel is a standard no. 3 scalpel handle. The no. 3 scalpel handle was chosen because it holds smaller scalpel blades then the no. 4 scalpel handle. It is possible to attach various compatible scalpel blades to this handle. Standard scalpels were used because they are easy to come by and commonly used in surgical procedures. The scalpel handle is clamped in the hollowed out end of a rod named the scalpel holder, an 8mm diameter stainless steel rod, with use of a screw. The diameter of the rod was set to 8mm to comply to the width of the standard scalpel handle. A screw was chosen to be able to detach the scalpel handle from the scalpel holder. It is possible to clamp the scalpel handle in such a way that movement relative to the scalpel holder is prevented. Although the screw protrudes from the rod, it does not interfere with the needed movement of the mechanism.

The other end of the scalpel holder has a threaded hole that fits the threaded end of the spring holder (M4), a stainless steel rod of 6mm diameter. A smaller diameter of 6mm was chosen for the spring holder in order to hold a broader selection of spring diameters. This way, multiple springs can be used in order to find the optimum impulse force. A stainless steel center disk is clamped between the two rods. This disk is needed to transmit forces from and to the spring. When the handle is pulled, the disk transmits the pulling force to the compression of the spring. When the handle is released the spring acts on the center disk in order to move the rod assembly that holds the blade. The disk has to transmit high forces from the spring, therefore the material is stainless steel and the center disk has a thickness of 2mm. The center disk has a flattened part that slides on the base in order to prevent rotation of the rod assembly and therefore keeps the blade in a horizontal position. The center disk is clamped between the two rods by a screw connection. A note should be made that rotation can only be prevented when the center disk is tightly secured between the two rods. The center disk has a diameter of 11mm. This diameter ensures enough surface for the spring to act on and it allows for a flattened part that slides on the base. The disk slides on a Teflon inlay in the base to minimize friction.

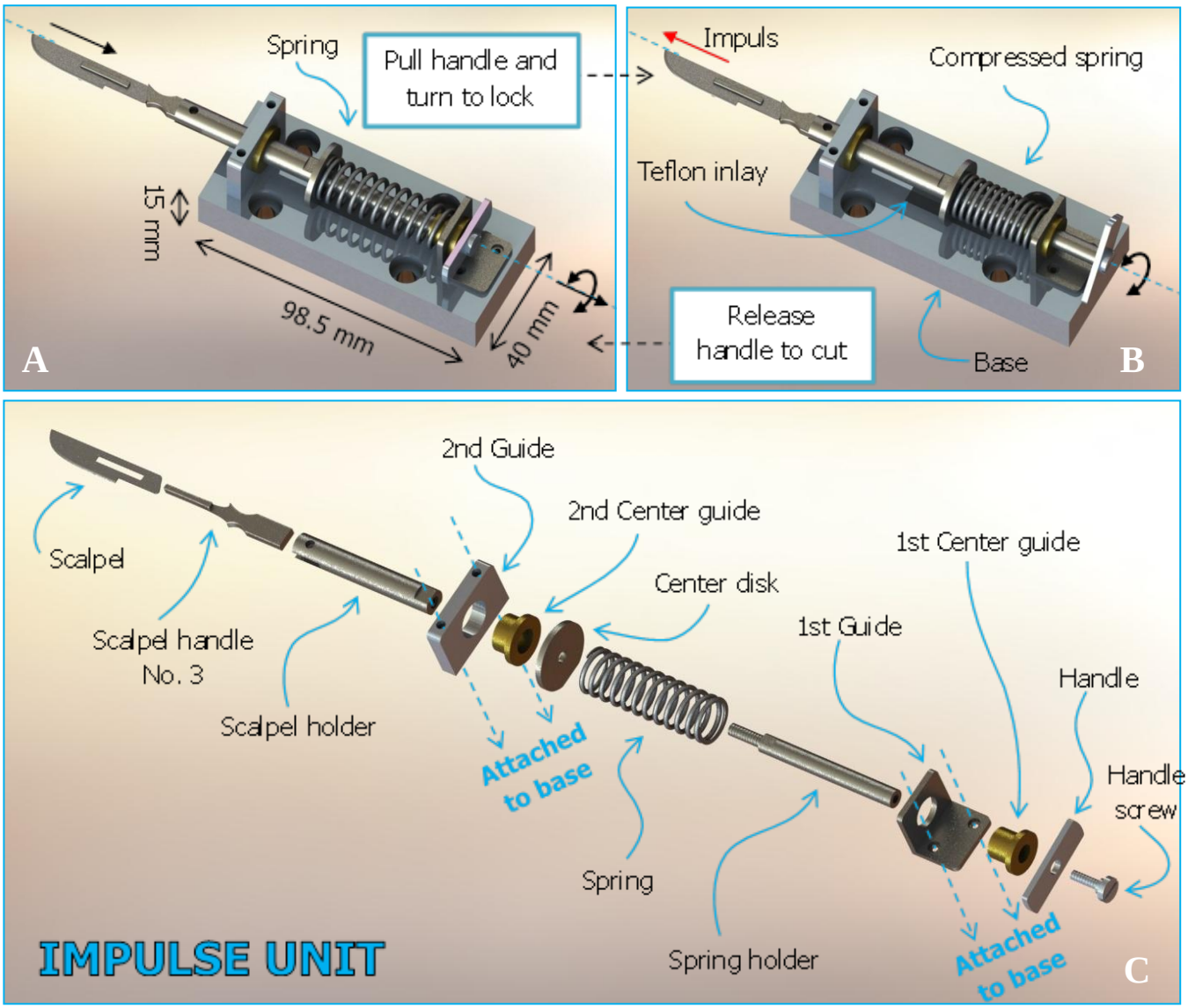


Figure 9: The idea of the impulse unit is that a sharp knife can be shot in a horizontal direction with great force. In order to comply with this idea, the impulse unit is built around a compression spring mechanism. The main rod assembly, that consists of a scalpel holder, a center disk, a spring holder and a handle, slides through two guides. A spring fits around the spring holder and is contained between the center disk and one of the two guides. When the handle is pulled in an outward direction the spring is compressed and stores potential energy. The handle can rotate and get caught by the base of the unit, hereby locking the spring in its compressed position. When the handle is rotated back to the free position, the energy is released and the spring pushes the rod in the opposite direction of pulling and an impulse is created. (A) rest state of the impulse unit. (B) compressed state of the impulse unit. (C) exploded view of the impulse unit minus the base.

The other end of the spring holder has a threaded hole that fits the handle screw (M3). The handle screw has a partially unthreaded shank below the head. The unthreaded portion has a bigger diameter and acts as the rotation center for the handle itself. The length of this unthreaded part is slightly bigger than the thickness of the handle. In this way, the handle is free to rotate without putting a rotational force on the rod assembly. The stainless steel handle has a rectangular shape with rounded edges with in the middle a circular hole of 5 mm. The rectangular shape is chosen in order to create a fine grip for the user; one finger on each side of the rod. When the unit is in rest state the short sides of the handle are aligned horizontally. After compressing the spring by pulling the handle outwards, the handle can be rotated to a vertical position. Part of the handle will hook behind the short side of the base, therefore locking the

mechanism in a compressed state. When the handle is turned to a horizontal position again, the spring pushes the rod assembly to its rest state.

The rod assembly slides through two guides that are attached to the base; the first guide and the second guide. The rods can slide more freely through the two aluminum guides due to the brass center guides. The movement is stopped by the handle reaching the center guide that is inserted in the first guide. The travelling distance of the knife is equal to the distance between the center guide and the edge of the base, this is 13mm, which is enough to cover the 8mm long mouth of the suction unit.

The aluminum, 2mm thick, first guide has an L-shape and is attached to the base by two countersunk screws. The vertical part has a circular hole with a diameter of 10mm that perfectly fits the first brass center guide. The first center guide has an inner dimension of 6mm, which is compatible to the diameter of the spring holder. The center guide has a head with a diameter of 14mm and a shank has a diameter of 10mm. The center guide is placed in such a way that, in rest state, the head of the center guide is clamped between the handle and the first guide. The other end of the center guide protrudes from the first guide and has the function of keeping the spring in place.

The second guide is placed on the other end of the base and is made of 5mm thick aluminum. It has a rectangular shape with rounded edges and in the middle a circular hole with a diameter of 12mm that perfectly fits the second brass center guide. It is attached to the base by two screws that go all the way through the top of the second guide into the base. The second center guide has an inner diameter of 8mm to fit the diameter of the scalpel holder. The head of the second center guide has a diameter of 16mm, and the shank has a diameter of 12mm. The two guides are attached to the aluminum base (98.5x40x15mm). In the middle of the base a rectangular hole has been made to fit a Teflon inlay (30x10x5mm). This Teflon inlay is placed in order to minimize the friction that is caused by the center disk moving on the base. The base also has four M6 countersunk screw holes that allow attachment to Thorlabs materials. It is important that the rod assembly can be disassembled. This disassembly is necessary to change the spring. The scalpel holder and the spring holder can be disassembled by means of the above described screw connection and flattened ends of the rod.

The spring that was used in the impulse unit had a spring constant of 2.03 N/mm, an equilibrium length of 47mm and a diameter of 13.5mm [41]. The scalpel blade that was used was a number 10, suitable for no. 3 scalpel blades. In Figure 10 a picture is shown of the scalpel blade.

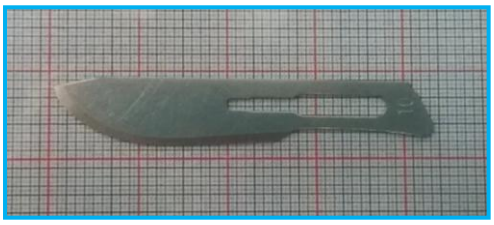


Figure 10: Picture of the used scalpel blade on graph paper. The red lines indicate 10mm.

2.3 Experimental set-up

The two units are combined to form the experimental set-up (Figure 11). The base of the set-up is a Breadboard from Thorlabs. Onto this Breadboard two Thorlabs Travel Translation Stages are mounted that function as the base for the impulse unit. Two Thorlabs Structural Rails are mounted onto the base plate with use of two Thorlabs 90° Mounting Adapters. The suction unit can slide vertically over the two Rails with use of M6 bolt and nut. The units are placed in such a way that the scalpel of the impulse unit in rest is directly covering the mouth of the suction unit. Alignment of the impulse unit in the x- and y-plane was reached by the two translation stages. The suction unit was aligned only in the z-plane using

the bolt and nut construction. A tube of 640mm long with an inner diameter of 2mm is attached to the suction unit. The other end of the tube is attached to a standard 50ml syringe. The syringe is placed in such a way that the handles of the barrel are in front of the two bolts that are mounted onto the Breadboard. Two extra bolts are mounted onto the Breadboard on the middle line of the two bolts behind the handles of the barrel. These bolts were used to hold the plunger in its place when suction was applied.

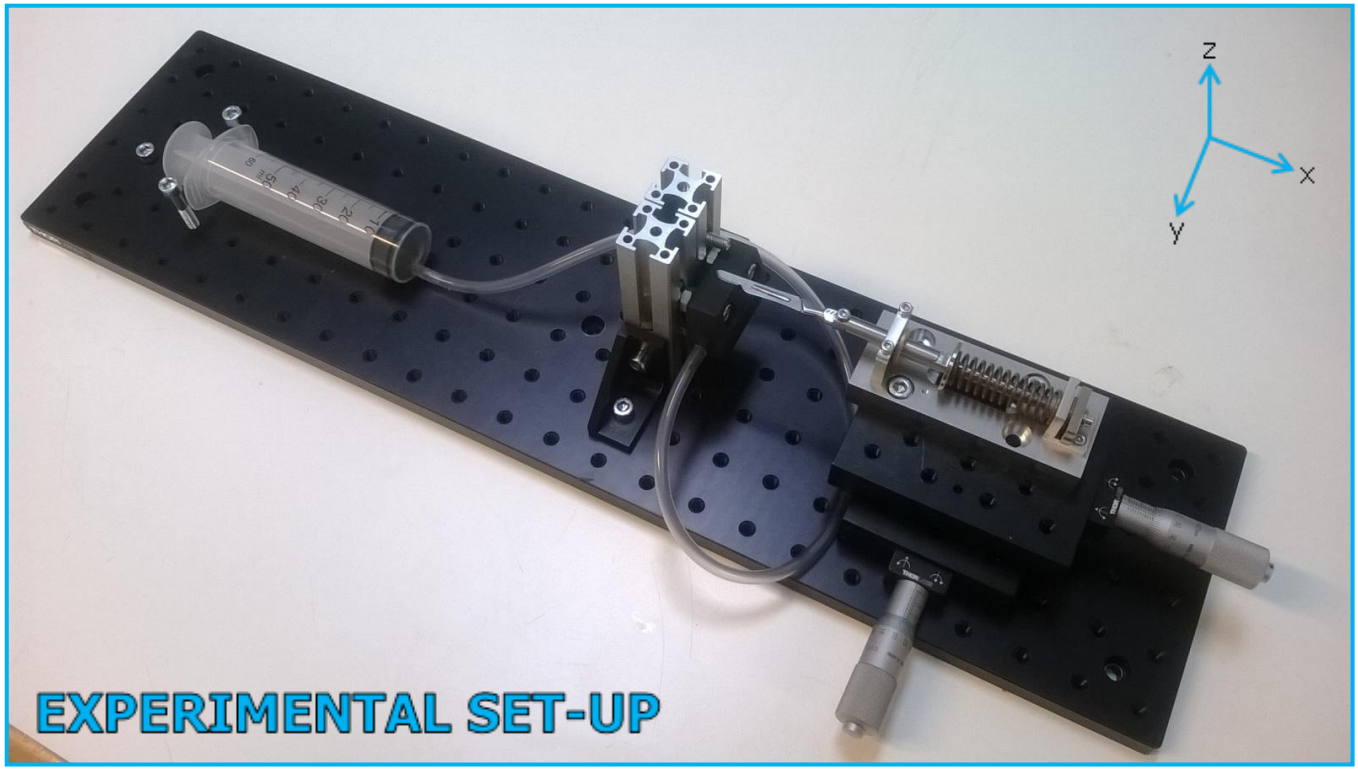


Figure 11: Picture of the experimental set-up. The base of the set-up is a Breadboard from Thorlabs. Onto this Breadboard two Thorlabs Travel Translation Stages are mounted that function as the base for the impulse unit. Two Thorlabs Structural Rails are mounted onto the base plate with use of two Thorlabs 90° Mounting Adapters. The suction unit can slide vertically over the two Rails with use of M6 bolt and nut. The units are placed in such a way that the scalpel of the impulse unit in rest is directly covering the mouth of the suction unit. Alignment of the impulse unit in the x- and y-plane was reached by the two translation stages. The suction unit was aligned only in the z-plane using the bolt and nut construction. A tube of 640mm long with an inner diameter of 2mm is attached to the suction unit. The other end of the tube is attached to a standard 50ml syringe. The syringe is placed in such a way that the handles of the barrel are in front of the two bolts that are mounted onto the Breadboard. Two extra bolts are mounted onto the Breadboard on the middle line of the two bolts behind the handles of the barrel. These bolts were used to hold the plunger in its place when suction was applied.

The general use of the set-up is as follows (Figure 12): upon use the scalpel is pulled back and the spring is compressed. The tissue is placed upon the suction mouth and the syringe is pulled to create a suction force. Then the spring is released and the scalpel uses the impulse to cut a slice in the shape of the mouth of the suction unit.

In the following sections the experimental set-up is described in more detail. To get an understanding of the used force and speed with which the tissue comes in contact the impulse characteristics are theoretically determined. The suction force is also theoretically determined.

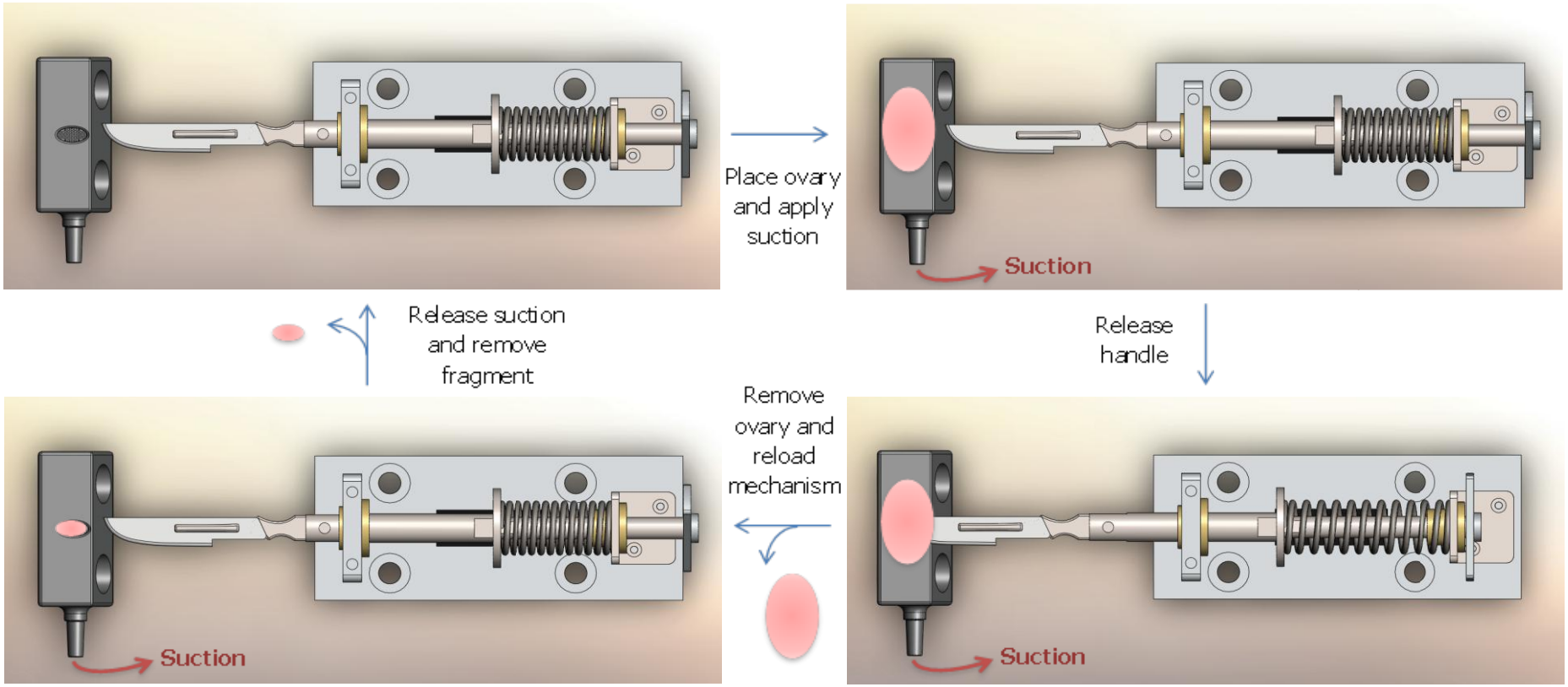


Figure 12: General use of the experimental set-up. upon use the scalpel is pulled back and the spring is compressed. The tissue is placed upon the suction mouth and the syringe is pulled to create a suction force. Then the spring is released and the scalpel uses the impulse to cut a slice in the shape of the mouth of the suction unit.

2.3.1 Theoretical impulse characteristics

In order to describe the dynamic behavior of the impulse unit, a standard mass-spring system was used as model. The impulse unit was designed to minimize friction. In combination with the high spring constant of the used spring it is thought that the friction force is negligible. The law of conservation of energy states that the total energy of a system remains constant over time. Friction is thought to be negligible, therefore the total energy of the system (E) consists of the potential energy (U) of the spring and the kinetic energy (K) of the moving rod assembly (equation 1). The potential energy is dependent on the spring constant (k = 2.03 N/mm) and the distance vector (x(t)) (equation 2). The distance vector describes the distance the spring is compressed from its equilibrium length over time (t). The kinetic energy is dependent on the mass (m = 48 g) of the rod assembly and the velocity vector (v(t)) (equation 3). The velocity vector describes the velocity at which the rod assembly is moving over time.

$$E = K + U = constant$$ (1) Law of conservation of energy

$$U = \frac{1}{2}k(x(t))^2$$ (2) Potential energy of a spring

$$K = \frac{1}{2}m(v(t))^2$$ (3) Kinetic energy

When the spring is locked in a compressed state, the potential energy is the only component adding to the total energy of the system because the kinetic component is zero. Upon release of the spring, the law of the conservation of energy states that the amount of potential energy that is lost is converted into kinetic energy. With an expression for the distance vector x, the velocity vector can be determined. Using Hooke's law and the second law of motion, the distance vector for an undamped mass-spring system can be written (equation 4), where x_0 is the compression of the spring at t=0.

$$x(t) = x_0 \cos(\sqrt{\frac{k}{m}}t)$$ (4) Distance vector

The momentum of the impulse unit (P) can subsequently be determined from the mass of the moving parts and the expression for the velocity derived from the law of conservation of energy (equation 5).

$$P = mv$$ (5) Linear momentum

In Figure 13 the dynamic behavior of the impulse unit is shown. Both the potential and the kinetic energy of the system is shown, as well as the course of the force, velocity and momentum of the impulse unit. The vertical dotted line represents the moment the scalpel comes in contact with the tissue sample on the suction unit. It can be seen that the velocity of the rod assembly is about 1.9 m/s. The force at which the scalpel reaches the tissue is about 26 N. The system still has more potential energy than kinetic energy and the momentum has not reached full potential. After this moment it is difficult to describe the dynamic behavior of the system because friction starts playing a bigger role as the scalpel makes contact with the tissue. Not only is there friction between the scalpel and the tissue sample, but due to the asymmetrical shape of the scalpel, the friction between the rod assembly and the two center guides will increase and this theoretical model will not be a suitable description.

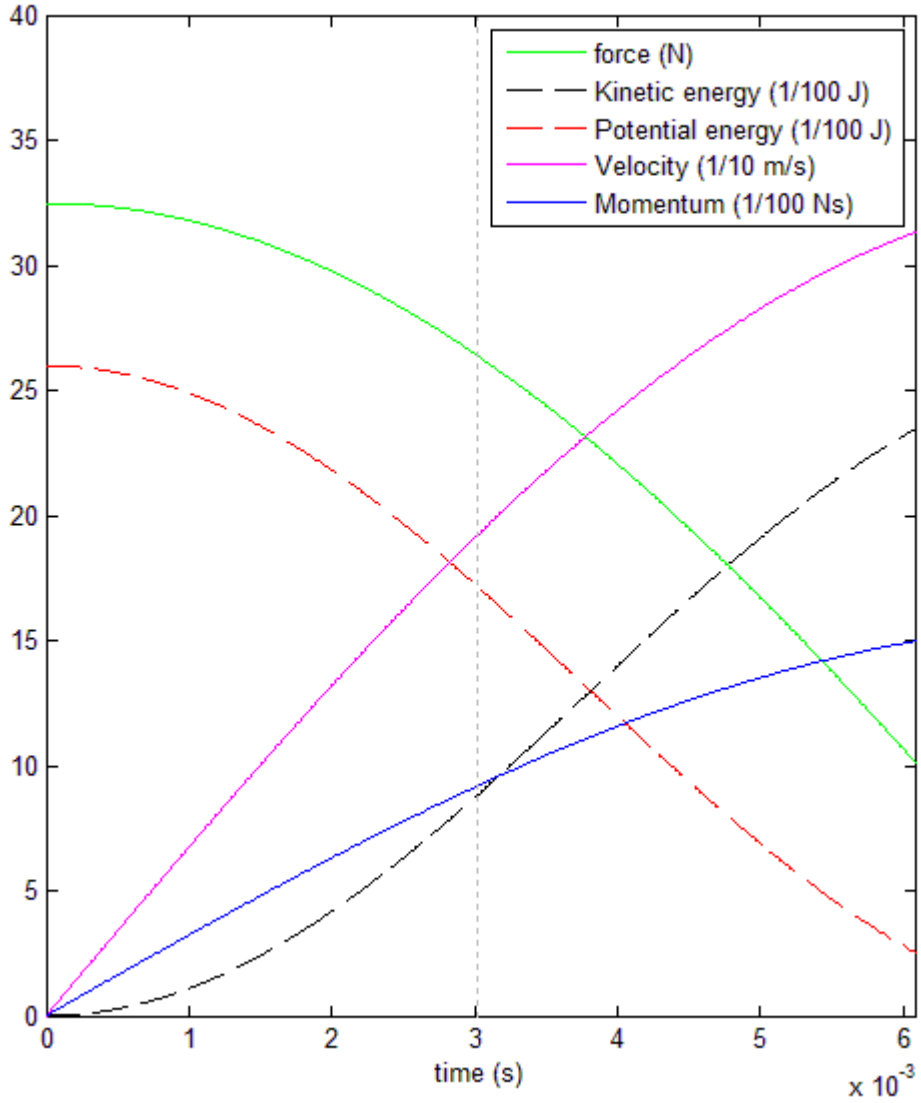


Figure 13: Dynamic behavior of the impulse unit. Both the potential and the kinetic energy of the system is shown, as well as the course of the force, velocity and momentum of the impulse unit. The vertical dotted line represents the moment the scalpel comes in contact with the tissue sample on the suction unit.

2.3.2 Theoretical suction force

The theoretical pressure difference and the theoretical suction force can be approximated with the standard atmospheric pressure and the closed off volume that is created by the suction unit, the tube and the syringe. According to the ideal gas law, pressure (p) depends on the closed off volume (v), the number of molecules in this volume (n), the ideal gas constant (R) and the temperature (T). Before suction is applied, the volume within the suction unit and the tube will be referred to as v1 and the pressure as p1. This pressure will be equal to atmospheric pressure, which is 100 kPa (equation 6). When pulling the syringe, the volume is increased (v2) but the number of molecules is not. This decreases the pressure in the closed off volume (p2), while the pressure in the space surrounding the suction unit remains the same. p2 can be described as the ratio of v1 over v2 times p1 (equations 7 – 9).

$$p_1 = \frac{nRT}{V_1} = 100\ kPa$$ (6) Pressure with closed plunger

$$V_1 = V_{tube} + V_{unit} = 11\ 230\ mm^3$$ (7) Approximate volume with closed plunger

$$V_2 = V_{tube} + V_{unit} + V_{syringe} = 19\ 230\ mm^3$$ (8) Approximate volume with extended plunger

$$p_2 = p_1 \frac{V_1}{V_2} = 58\ 000\ N/m^2$$ (9) Approximate pressure with extended plunger

The difference in pressure (Δp) between p1 and p2 can be used to calculate the suction force (equation 10). Pressure is determined as the force applied perpendicular to the surface of an object per unit area over which the force is distributed. In the case of the suction unit, the suction force F can be expressed as the pressure difference times the total area (A) of the holes in the grid of the suction mouth. This comes to a suction force of 1.6 N (equation 11).

$$\Delta p = 42\ 000\ N/m^2$$ (10) Approximate pressure difference

$$F_{suction} = \Delta p * A_{mouth} = 1.6\ N$$ (11) Approximate suction force

2.4 Tissue

For the experiments different types of tissue were used to determine the feasibility of the concept technique for harvesting ovarian tissue. The origin of these tissue types are described in the following sections.

2.4.1 Chicken tissue

The experiments were firstly performed with tissue originating from chickens. Two different tissue types were used, namely chicken filet and chicken heart. The tissue samples were obtained from supermarkets and butchers. Chicken filet is a tissue type that is very different from ovarian tissue, mainly because it is not a whole organ. Chicken heart is an organ just like the ovaries. This means that the surface is covered with a membrane. The chicken hearts were therefore only used on the outside of the organ, in other words, the part that is covered with this membrane.

2.4.2 Ovarian tissue

Ovaries were obtained of bovine origin (Figure 14). Bovine ovaries have overlap with human ovaries in terms of volume and in terms of the monthly cycle. In a study the volume of human ovaries was compared with ovaries from 3 other species, namely bovine, porcine and ovine ovaries (Figure 15). It can be seen that porcine ovaries correspond the most with human ovaries. Bovine ovaries have a larger range in volume than human ovaries, but they do overlap. Ovine ovaries have much smaller volume compared to human ovaries.

Figure 14: Picture of a bovine ovary and a paper teabag for scale.

Although porcine ovaries correspond the most with human ovaries in terms of volume, the monthly cycle of porcine differs: multiple follicles mature each month. In human and bovine ovaries ordinarily only one follicle matures [42]. The number of maturing follicles is of influence on the characteristics of the cortex of the ovary. Maturing follicles bulge out of the ovary, and change the shape and composition of the cortex. Therefore bovine ovaries are a more adequate model for human ovaries.

The bovine ovarian tissue was obtained via the Leiden University Medical center from a slaughterhouse. The ovaries were preserved in a medium. This medium contained three active substances, namely; one to increase vasodilation, an anticoagulant, and antibiotics. These substances could influence the tissue characteristics. In all experiments the ovaries were used as whole organ.

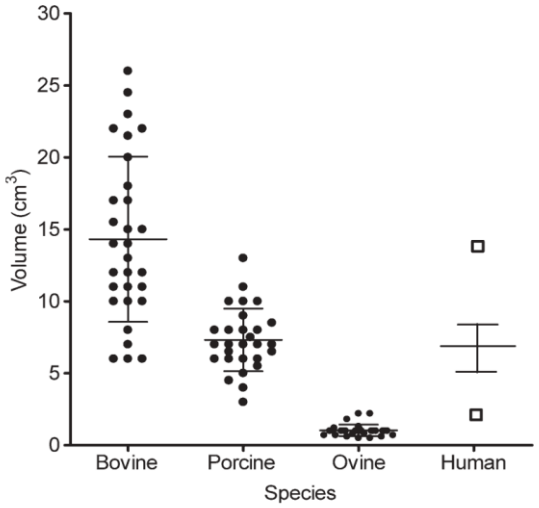


Figure 15: Volume of the ovaries for different species. Each solid circle represents an individual measurement, and mean volumes ± standard deviation are indicated [42].

2.5 Experiments

In the following sections the methods for several experiments are explained. Firstly the set-up was used to determine the applied suction force with two tissue types. Subsequently, the experimental set-up was tested with several tissue types to get a global idea about the efficacy of the concept technique. Finally, an experiment was done to determine the extent to which the experimental set-up can provide standardized fragments. For this experiment the length and the width of the obtained fragments were measured.

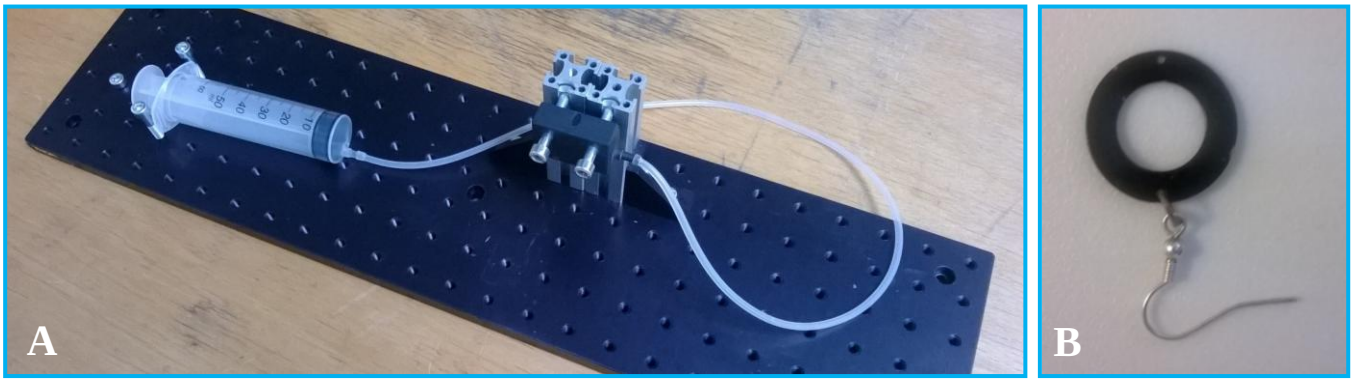


Figure 16: (A) Experimental set-up used to measure suction force. The suction unit was tightly secured to the top of two Thorlabs Structural Rails with bolt and nut, which in place were attached onto a Thorlabs Breadboard with use of two Thorlabs 90° Mounting Adapters. A tube with 2mm inner diameter was attached to the suction unit while the other end was attached to a 50ml syringe. The syringe was placed in such a way that the handles of the barrel were in front of the two bolts that were mounted onto the Breadboard. Two extra bolts were mounted onto the Breadboard on the middle line of the two bolts behind the handles of the barrel. These bolts were used to hold the plunger in its place when suction was applied. (B) The earring hook used to attach the tissue sample. The ring was used to pull the tissue sample from the suction unit.

2.5.1 Experiment suction force

Besides a theoretical value, the suction force was also measured. Measuring was done with use of the set-up in Figure 16. The suction unit was tightly secured to the top of two Thorlabs Structural Rails with bolt and nut, which in place were attached onto a Thorlabs Breadboard with use of two Thorlabs 90° Mounting Adapters. A tube with 2mm inner diameter was attached to the suction unit while the other end was attached to a 50ml syringe. The syringe was placed in such a way that the handles of the barrel were in front of the two bolts that were mounted onto the Breadboard. Two extra bolts were mounted onto the Breadboard on the middle line of the two bolts behind the handles of the barrel. These bolts were used to hold the plunger in its place when suction was applied. For the experiment the syringe was used to increase the volume with 8ml by looking at the volume indicting marks on the barrel. The experiment was performed with chicken filet and bovine ovaries. For chicken filet four tissue samples were used to take twenty measurements. For bovine ovary one tissue sample was used to take ten measurements. The suction force was determined by measuring the force needed to pull the tissue sample loose from the suction unit. Figure 17 shows a schematic force analysis of the tissue fragment during the suction force experiment. The tissue samples were attached to an earring hook that was connected to a ring. A balance (Kern EMB 600-2) with a hook attached to the bottom of the balance was connected to the ring. Firstly, the weight of the tissue sample together with the earring hook was measured in order to determine the gravitational mass (m_g). The gravitational force is the weight times the acceleration of gravity (g = 9.81 m/s^2). Subsequently, the tissue sample with ring was placed on top of the suction unit and suction was applied. The hook of the balance was attached to the ring of the tissue sample. By moving the balance in an upward direction by hand, the tissue sample was pulled from the suction unit (F_{pull}). The maximum weight that was displayed on the balance resembles the force needed to pull the tissue from the suction unit (m_{pull}). This is a combination of the suction force ($F_{suction}$) and the gravitational force (F_g). For the complete protocol of the performed experiment see appendix I. The obtained data was submitted to Matlab. The suction force was calculated according to equation 12.

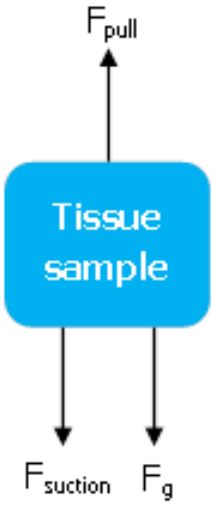


Figure 17: schematic force analysis of the tissue fragment during the suction force experiment. F_{pull} is the force that is needed to pull the tissue sample from the suction unit. $F_{suction}$ is the suction force and F_g is the gravitational force of the tissue sample.

$$F_{suction} = (m_{pull} - m_g) * g \qquad \text{(12) Suction force}$$

2.5.2 Experiment tissue types

The experimental set-up was used with different tissue types to obtain a global idea about the efficacy of the concept for a harvesting technique. Three types of tissue were used in this experiment, namely chicken filet, chicken heart and bovine ovaries. The set-up described in section 2.3 was used for this experiment. Firstly, the impulse unit was put in a compressed state. For each tissue type, the tissue sample was placed on top of the suction unit after which suction was applied by using a syringe to increase the volume with 8ml. Subsequently, the handle of the impulse unit was released to create an impulse. The full protocol is described in appendix II. The exact number is tests was not tracked, but an approximation of the number of tests is as follows: for this experiment ten chicken filet tissue samples

were used to perform around forty-five tests, three chicken hearts were used to perform around fifteen tests and two bovine ovaries were used to perform the test around ten times. For each tissue type it was determined whether the experimental set-up was successful in entering the tissue and in cutting a complete fragment. With entering tissue is meant that the scalpel is inside the tissue sample. With cutting a complete fragment is meant that the fragment is completely separated from the tissue sample that was placed on top of the suction unit. The complete fragments that were obtained during this experiment were used to in the experiment to determine fragment dimensions, section 2.5.3.

2.5.3 Experiment fragment dimensions

In order to determine the accuracy and the precision of the experimental set-up the length and width of obtained fragments were measured in this experiment. The length and width were analyzed separately. In this way one variable is in line with the impulse force, while the other variable is perpendicular to the impulse force The fragments obtained from the tissue type experiment, section 2.5.2, were used in this experiment. Fragments were obtained from chicken filet and chicken heart. The experimental set-up on its own, was unable to cut complete fragments of heart tissue. However, fragments were still obtained by pulling the tissue horizontally towards the opposite direction of the blade side of the scalpel. On the other side of the fragment the membrane was severed using a separate knife. For this reason the width of the heart tissue fragments will not give correct information about the accuracy of the set-up. Therefore, it will not be analyzed. Immediately after the fragment was cut, the fragment was placed on a sheet of laminated graph paper with 0.5mm accuracy [43]. The fragments were positioned in such a way that the length is parallel to the lines of the graph paper. The length and width of the sliced fragments are measured using a microscope (25X – 200X USB Digital Microscope) that was connected to a laptop. The program VLC media player was used to display the image from the microscope. For each fragment the image was saved and analyzed.

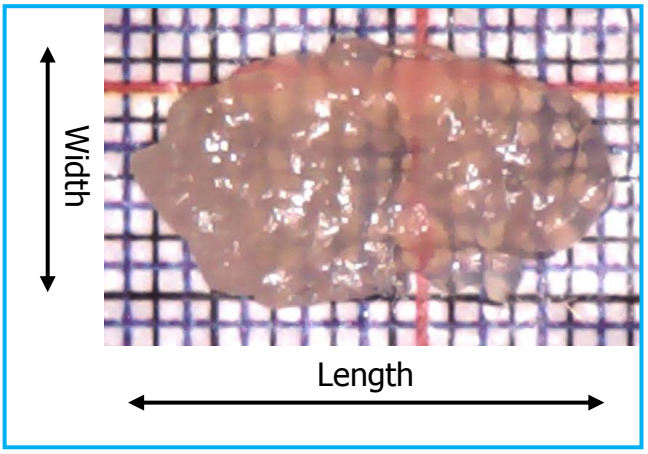


Figure 18: Example of tissue fragment that resulted from the use of the experimental set-up. The tissue fragment is placed on top of laminated graph paper with a line distance of 0.5mm. The length and width of the fragment are shown.

The length and width were measured individually by hand. Figure 18 shows an example of a tissue fragment. For the complete protocol see appendix III. The obtained data was analyzed with Matlab. For both the length and width of the fragments, the median, the extremes and the 25^{th} and 75^{th} percentile were determined. The median is equal to the value of the number that divides the higher half from the lower half in a data set. The extremes are the minimum and the maximum value of the data set. Percentiles correspond to the value below which a given percentage of observations fall. In order to determine the accuracy of the set-up the difference between the intended value and the median was compared to the intended value. Results were considered accurate if the difference was 10% or less. The precision of the set-up was determined by comparing the difference between the 25the and 75the percentile to the median. Results were considered precise if the difference was 10% or less.

The length of the chicken filet fragments was compared with the length of the chicken heart fragments in a boxplot. The notches display the variability of the median between samples. When notches do not overlap, the medians of the two boxplots are significantly different with a 5% significance level. This significance level is based on a dataset with a normal distribution, however, its holds for other distributions as well. This way of comparing medians is analogous to using a t-test for means [44]. Additionally, a two-sample t-test was performed to obtain the p-value of the two independent data sets.

3 Results

The experimental set-up was used to perform several experiments. First, the actual suction force was measured using chicken filet and bovine ovaries. Secondly, several tissue types were used to determine the global efficacy of the set-up. Finally, chicken filet and chicken heart were used to determine the accuracy and precision of the set-up by measuring the fragment length and width. In the following sections the results from these experiments are shown.

3.1 Suction force

The suction force was measured for comparison to the theoretical value that was calculated in section 2.3.2. The force was measured for chicken filet and bovine ovaries. In Figure 19 the obtained values are shown separately for each tissue type. It can be seen that the values for chicken filet range between 0.2 – 1.1 N. The values for bovine ovary range between 0.2 – 1 N.

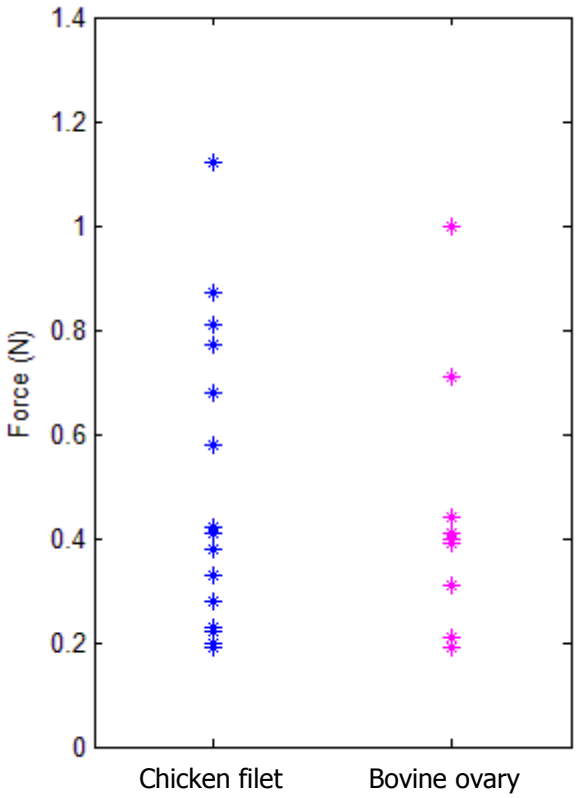


Figure 19: Variability of the suction force measurements with chicken filet and bovine ovary.

3.2 Tissue types

In order to get a global idea about the efficacy of the experimental set-up several tissue types were used in an attempt to obtain fragments. In Table 4 the types are described together with the number of distinctive tissue samples were used and the number of tries that was made to obtain a fragment. For each tissue type is also stated whether it was possible for the scalpel to enter the tissue. With entering tissue is meant that the scalpel is inside the tissue sample. It is also stated whether the experimental set-up was successful in cutting a complete fragment, this means that the fragment is completely separated from the tissue sample. It can be seen that the experimental set-up was successful in cutting fragments of chicken filet. With chicken heart, the set-up was successful in entering the tissue, but a complete fragment could not be reached. The set-up was unsuccessful with bovine ovaries. Entering the ovaries was not possible with the set-up.

Table 4: Results of several tissue types used with the experimental set-up. The # of samples refers to the number of distinctive tissue samples, the # of tests refers to the number of tries that was made to obtain a fragment with the experimental set-up.

Tissue type	# of samples	# of tests	Successful in	
			Entering tissue	Cutting fragment
Chicken filet	± 10	± 45	Yes	Yes
Chicken heart	3	± 15	Yes	No
Bovine ovaries	2	± 10	No	No

3.3 Fragment dimensions

In order to determine the accuracy and the precision of the experimental set-up the fragments obtained using chicken filet and chicken heart were measured. The experimental set-up was unable to cut complete fragments of heart tissue. However, fragments were still obtained by pulling the tissue horizontally towards the opposite direction of the blade side of the scalpel. On the other side of the fragment the membrane was severed using a separate knife. For this reason the width of the heart tissue is not representative of the experimental set-up and therefore it was not analyzed. Length and width were separately analyzed, the results are shown in Table 5 and Figure 20.

It can be seen in Table 5:Characteristics of the length and width of the tissue fragments obtained with the experimental set-up. that for chicken filet the median value for the fragment length is slightly shorter than the length of the mouth from the suction unit, respectively 7.5mm and 8mm, while the value for the fragment width is slightly larger than the width of the mouth from the suction unit, respectively 4.5mm and 4mm. The difference between the measured median and the intended dimension of the mouth is as follows. For the length of chicken filet fragments this is 6.25%. For the width of the chicken filet fragments this is 12.5%. And for the length of the chicken heart fragments there is no difference. The difference between the 25^{th} and the 75^{th} percentile show that for the length of the chicken filet fragments, 50% of the data samples lie within 0.75mm difference. This is 10% of the median value. For the width of the chicken filet fragments, 50% of the data samples lie within 1mm difference. This is 22% of the median value. For the length of the chicken heart fragments, 50% of the data samples lie within 0.6mm difference. This is 7.5% of the median value.

Table 5:Characteristics of the length and width of the tissue fragments obtained with the experimental set-up.

Characteristics	Chicken filet (40 fragments)		Chicken heart (9 fragments)
	Length (mm)	Width (mm)	Length (mm)
Median	7.5	4.5	8
Minimum	6	4	6
Maximum	8.5	6	8.5
25th percentile	7	4	7.4
75th percentile	7.75	5	8

When comparing the length of the fragments obtained from chicken filet with chicken heart the same variability is seen. The values range between 6mm and 8.5mm. The median is, however, larger for chicken heart. In Figure 20 it can be seen that the median for chicken heart has a higher value than the median of chicken filet. It also shows that the 75th percentile has the same value as the median of the chicken heart data set. The red dotted line indicates where the notch of one boxplot ends and the notch of the other boxplot begins. The two independent data sets were also submitted to a two-sample t-test. The resulting p-value had a value of 0.2.

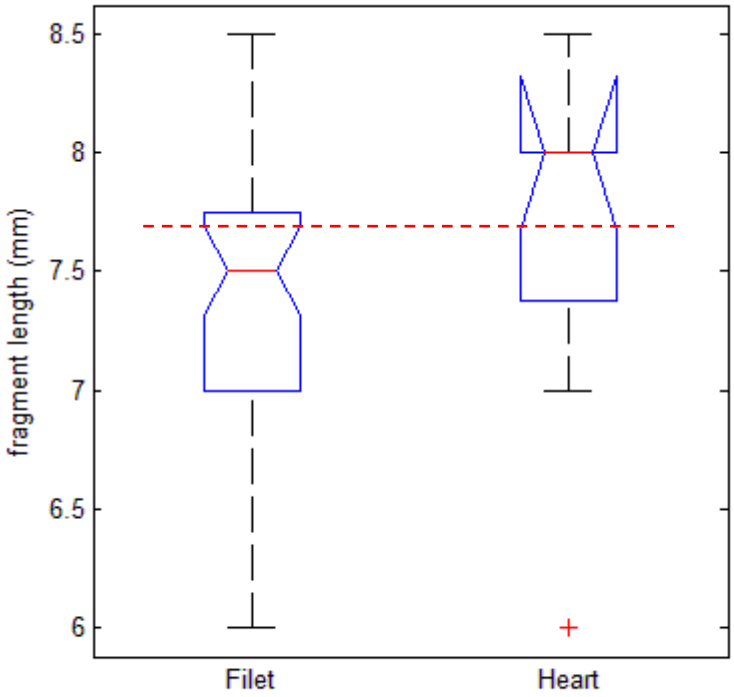


Figure 20: Boxplot of the length of the tissue fragments obtained with the experimental set-up and two types of tissue, namely chicken filet and chicken heart. The red line indicates the median of the data set and the top and bottom line of the box indicate the 75th and 25th percentile. The notches display the variability of the median between samples. The red dotted line shows the border of the notches for both boxplots.

4 Discussion

4.1 Design of units

To create the set-up from two separate units gave the opportunity to really focus on their function in the design and leave alignment to separate specialized tools, such as the translational stages from Thorlabs. Upon use, the units functioned according to the expectations, however, some aspects were discovered that could be improved. These aspects will be described in the following sections.

4.1.1 Suction unit

The size determining feature of the suction unit was the screw holes that would allow for attachment to Thorlabs materials. In order to not increase the size, and therefore the production costs of the suction unit, the screw holes were dimensioned exactly to an M6 screw. However, the suction unit was 3D-printed in two parts and then glued together. Therefore, the accuracy was changed which resulted in screw holes that barely fitted M6 screws. The screws did not fit the holes without having to screw them in and leaving threaded marks on the inside of the holes. This did not oppose a real problem because the wall of the suction unit was chosen to be thick enough. Attaching and detaching the suction unit to and from the structural rails was a bit more difficult than expected, because more force was needed to secure it tightly. However, this feature also has an advantage: it was possible to attach the suction unit in a stable and secure way while using long screws that would leave space between the rails and the suction unit itself. This setting was used during the suction force experiment.

A second remark on the design of the suction unit is the choice for a slope on the front side of the box to allow easy access to the mouth. Due to the flexibility of the scalpel and its position onto the scalpel handle, the scalpel would not always reach the suction unit on the same vertical position. A few times it occurred that the point of the scalpel was cutting into the edge of the mouth instead of sliding directly over it. For the experiments with chicken filet this problem was circumvented by attaching the suction unit in the opposite direction. That way the scalpel could slide a small distance over the top of the suction unit before reaching the mouth.

After using the suction unit, the mouth needed to be cleaned. The depth of the grid was equal to the thickness of the wall of the suction unit, which was 5mm. This created some problems with cleaning because it was difficult to remove small pieces of tissue that had entered the grid. This issue was resolved by using the syringe to blow air or water through the grid from the inside out. A toothbrush was used to clean the surface of the mouth.

4.1.2 Impulse unit

The impulse unit was built around a spring mechanism. In order to make the spring interchangeable, the main rod assembly consisted of two parts that were screwed in together, namely the scalpel holder and the spring holder. Between the parts, a center disk was clamped to prevent rotation and to serve as containment of the spring. Both the scalpel holder as the spring holder have a flattened end that can be used to detach the two parts with two wrenches. However, the spring that was used for the experiments had a high spring constant. In order to reach the flattened part of the spring holder the spring must manually be compressed to be able to get the wrench in the right position. It was possible to get the wrench in the right position but it could be improved in a next design. For instance, by extending the spring holder in a way that it protrudes from the end of the base, where the handle resides. This part of the rod could then be flattened to create the grip for the wrench. In this way, the spring would not cover the wrench grip, but it would be freely accessible for the wrench.

The high spring constant had another consequence. The first guide was made from aluminum. When the handle was released, it would scrape the edge of the first guide. The handle was made from stainless steel, the high force of the spring would cause the edges of the first guide to splinter. The impulse unit would be greatly improved if the first guide was made from stainless steel as well. As added benefit, the thickness of the first guide could be reduced. Another way to improve the first guide would be to zinc it into the base in such a way that the surface of the base is equal to the surface of the first guide. In this way the handle would not pass the edge of the first guide.

A last remark about the impulse unit has to do with the alignment of the scalpel. While the center disk has a flattened part to prevent rotation of the scalpel, the attachment of the scalpel to the scalpel handle was not taken into account. This attachment was not completely fixed. The scalpel could make a minimal rotation around the long axis. In a next design the attachment of the blade could be improved on this front.

4.2 Experimental set-up

For the experimental set-up the suction unit and the impulse unit were combined on top of a Thorlabs breadboard. The two translational stages and the structural rails were effective in reaching the right alignment of the two units. The translational stages use a spring mechanism in order to have a translation as result. This spring had a smaller spring constant than the spring used in the impulse unit. Upon loading the impulse unit, it was needed to hold the translational stage that was oriented in the same direction as the impulse unit. Otherwise the spring in the translational stage would compress while pulling the handle of the impulse unit, making it more difficult to lock the handle behind the base.

While using the set-up the scalpel blade was changed every four cuts. For safety reasons the scalpel was only changed with the impulse unit in rest state. In this state the scalpel is covering the mouth of the suction unit, therefore one of the units had to be removed. Moving the impulse unit in the x-direction with use of the translational stage did not create enough space to remove the scalpel blade. Loosening the suction unit and moving it down was effective, but the orientation of the suction unit changed slightly every time it was fastened. This is not ideal during an experiment. Therefore, it was chosen to remove the impulse unit itself. The impulse unit was attached to the translational stage with two screws

positioned diagonally opposed to each other. The impulse unit was designed with four screw holes, however, only two were used to speed up the process of changing the scalpel blade. Two screws were enough to create a stable attachment to the translational stage.

The suction unit was attached to the structural rails with two screws. This created a stable positioning for the suction unit. As stated before, the orientation of the suction unit changed slightly every time the unit was loosened and fastened. Much consideration was given to the placement of the suction unit. First, the suction unit was placed in a low position onto the rails. Subsequently, the impulse unit with scalpel blade was positioned. Then, the suction unit was moved in an upward direction until the blade was directly on top of the suction unit. In this position the suction unit was fastened. However, due to small changes during the fastening of the suction unit and the small freedom of movement of the scalpel blade, the alignment was not always the same.

Attached to the suction unit was a tube. The other side of the tube was attached to a standard 50ml syringe. The way the syringe was secured to the breadboard leaves something to be desired. Two screws hold the hold the handles of the barrel of the syringe while a third screw is placed to hold the plunger when suction is created. This method was successful in creating the same volume difference for each test. However, one hand was needed to hold the syringe in place. More attention could have been given to a proper solution for securing the plunger relative to the barrel of the syringe.

4.2.1 Suction force

In section 2.3.2 the theoretical value for the suction force is calculated, this is 1.6N. This value is not exact due to some practicalities. In the calculation the assumption is made that the tube will not deform under the pressure difference. In reality this will occur, thereby resulting in a smaller volume difference and therefore a smaller suction force. Another volume changing factor that is not included in the calculation is the suction mouth. To what extent has the tissue sample filled up the mouth of the suction unit before applying suction. If the tissue sample does not fill up the mouth but is in line with the top surface of the suction unit, v1 will have a different value. Therefore the pressure difference will differ, as well as the suction force. The volume of the mouth will add a maximum of $100mm^3$ to the total volume. The volume difference, created by the syringe, is measured in millimeters accuracy (1ml = $1000mm^3$). This measurement is not very accurate, therefore the difference in volume due to the volume of the mouth is thought to be insignificant.

Due to the small open area of the mouth, the final suction force is very low while the pressure difference is high. This is calculated to be 42 kPa. This pressure difference is comparable to the Octopus Tissue stabilizer, which reaches a pressure difference of 53 kPa [40]. For the Octopus Tissue stabilizer, this pressure difference is enough to stabilize part of the heart. However, it uses multiple compartments on the heart. The force could be increased if the area of the holes of the mouth could be increased, or if the mouth itself could be increased. Increasing the area of the holes can be done by using a different production technique or a more accurate 3D-printer that allows for smaller spacing between the holes. The area of the holes could also be increased by using less, but bigger holes. The downside of this option is that bigger holes increase the amount of tissue that could be sucked into the suction unit.

An experiment was done to measure the actual suction force that was reached with the suction unit. The results of this experiment are shown in Figure 19. It can be seen that the measured force for both tissue types has a high variability. The highest and the lowest value differ a factor five. The theoretical suction force of 1.6 N is not reached in this experiment. The variability of the measured values can be explained as follows. The method of measuring has some flaws. For instance, the positioning of the tissue sample on the suction unit relative to the inserted hook. When not in line on the z-axis, pulling the hook would result in a force direction that is not perpendicular to the surface of the mouth. Less force is needed to pull the tissue from the suction unit when it is not perpendicular. However, the use of this technique in a clinical setting could also result in non-perpendicular pulling of the tissue sample.

Another explanation for the variability of the measured values is the manner in which the suction is applied. The controlled variable in the experiment is the amount of ml that the plunger is pulled from the syringe. It could be that the tissue that was placed on the mouth, did not seal off the mouth completely before applying suction. Upon the start of suctioning a small whiff of air could enter the suction unit before the tissue completely sealed off the mouth. This whiff of air would change the amount of molecules in the suction unit and therefore the pressure difference and the suction force. It can be concluded that the described method of applying suction does not lead to precise values. It would have been better to use force as controlled variable for the experiment. When the force needed to pull the plunger would be constant, the suction force would be constant. In this case, the measured variability of the suction force would be a result from flaws in the method of measuring the suction force, like the placing of the hook. This would determine the accuracy of the measuring method. The reason to perform the experiment in this way has to do with the practical application in the operating room. When using a syringe to apply suction it is much easier to pull and stabilize the plunger after a certain amount of milliliters than stabilizing the plunger when a certain force is reached.

4.2.2 Tissue types

To test the global efficacy of the experimental set-up, several types of tissue were used with the set-up. The set-up was successfully tested with chicken fillet. From this test experiment it can be concluded that the principle of the concept works. However, chicken fillet is quite different from ovarian tissue. The second tissue type that was used is chicken heart. This tissue resembles ovaries better because it is also a complete organ. All organs are covered with a membrane. This membrane is very thin but strong. With heart tissue it was possible to puncture the membrane and enter the tissue but the sides of the fragment were not completely severed from the heart itself. The scalpel that was used is asymmetrical. Only one side of the scalpel blade could cut. The side of the fragment that was located on the cutting side of the scalpel could easily be severed by pulling the heart horizontally in the opposite direction of the cutting side of the scalpel. The other side of the fragment could not be severed with the set-up because the scalpel had no blade on that side. This could be solved by using a scalpel blade with two cutting sides. The final test with bovine ovaries gave poor results. The set-up was not able to even enter the tissue.

The efficacy of this set-up is proven, however not with the type of tissue for which the set-up was developed. Could the set-up create ovarian fragments if some of the parameters changed? In the following paragraphs an analysis is made from the set-up in order to understand why it is not successful in making ovarian tissue fragments.

A possible explanation is that the membrane surrounding the ovaries is even stronger than that of the heart, or there is a difference between the animals that the tissue originated from. It could be possible that the serous membranes of bovines are stronger than those of chickens. Besides looking at the membrane, the organ itself could be compared. A heart mostly consists of muscle tissue, while an ovary is made up out of blood vessels and a cortex with egg cells. When comparing the heart tissue with the ovarian tissue a difference could be felt in the stiffness of the material. The chicken heart felt much stiffer than the bovine ovaries. for the purpose of visualization, ovarian tissue can be compared with a balloon filled with water. This means that the ovary is very deformable. It could also be said that the ovary has lower inertia than the heart. Inertia is partly dependent on the mass of the object. The heart tissue was not weighted, therefore a conclusion cannot be made on this part. However, a lower inertia could be compensated with a higher momentum. Figure 13 shows the dynamic characteristics of the impulse unit. It can be seen that it has not reached full momentum in the current configuration of the impulse unit. Full momentum is reached when the potential energy of the system is zero. This means that the spring has reached its equilibrium length. If the scalpel would reach the tissue sample at this moment the spring would have to stretch to actually enter the tissue. This could only occur when the impulse unit was designed in a way that would allow a harmonic motion of the spring. The design of the impulse unit is not made for harmonic motion because the spring is not attached to the impulse unit. It can only move the moving parts of the impulse unit by pushing the center disk. It cannot pull the center disk. A higher momentum could also be reached by using a spring with a higher spring constant. However, using a stiffer spring would make manual control of the impulse unit quite difficult.

Besides the effect of the impulse unit on the ovary, the suction unit should be considered. The ovary is highly deformable. When suction is applied and the scalpel reaches the tissue sample, the deformability of the ovary will result in a change in shape. If the part of the ovary that resides in the mouth deforms, the suction force is released because air can enter the suction unit. The suction force should be high enough to stop deformation of the tissue in the mouth. Unfortunately, the suction force is very low. A way to overcome this problem is to create multiple suction compartments that can be separately operated. In this way, not the complete suction force will fall away, but only an compartment. A second solution will be to use active suction. This will keep pulling the ovary into the mouth even when it deforms. For future research even a combination of the two could provide the optimum solution. It can however be concluded that the current way of applying suction is not effective for cutting ovarian fragments.

When the architecture of the mouth is considered, the shape of the wall will influence the angle at which the scalpel reaches the tissue. In the current design, the wall of the mouth flows fluently into the top surface of the suction unit. There is no perpendicular transition from the surface of the suction unit to the wall of the mouth. If the shape of the wall would be turned around, there would be a perpendicular transition between the wall and the surface of the suction unit and a fluent transition from the wall to the recessed surface of the mouth. When suction is applied, tissue would be pulled into the mouth and at the border of the mouth the tissue would be perpendicular to the surface of the suction unit. In this case the scalpel of the impulse unit will reach the tissue in a perpendicular way, which could increase the possibility of the scalpel entering the tissue.

4.2.3 Fragment dimensions

In order to determine the accuracy and the precision of the experimental set-up, the length and width of the obtained tissue fragments were measured. It can be seen in Table 5 that for chicken filet the median values for length and width differ from the dimensions of the mouth of the suction unit. The difference in fragment length can be explained by looking at the impulse direction. The knife does not only cut the tissue but also pushes it slightly forward, resulting in a fragment length slightly less than the actual dimensioning of the mouth. The difference in fragment width can be explained by looking at the alignment of the suction unit and the width and flexibility of the scalpel used in the experiment (Figure 10 shows a scalpel width of 8mm). The attachment of the scalpel to the scalpel handle is not completely fixed, it can make a minimal rotation around the long axis. In the alignment of the suction unit only the height (z-axis) can be adjusted. Possible rotation around the x-axis or y-axis cannot be compensated. These rotations differ every time the suction unit is attached to the rails. This means that the scalpel will not level out completely with the top surface of the suction unit. The scalpel itself is a thin piece of metal that is quite flexible and can bend when small forces act upon it. Additionally, the scalpel is much wider than the mouth of the suction unit. When the scalpel is shot in the impulse unit and it reaches the tissue sample, the flexibility of the scalpel may cause the scalpel to bend slightly upwards and the width of the scalpel causes the unit to cut fragments that are wider than the mouth of the suction unit.

Another factor to include is the flexibility of the tissue. The flexibility plays a role when suction is applied but also when the tissue fragment is taken from the mouth of the suction unit and put onto the laminated graph paper. The fragment is placed in such a way that the lines on the graph paper are in line with the length and width of the fragment. This placement can result in stretching of the fragment which again can result in larger or smaller measurements. This flexibility implies that the results should be seen as a global measurement and cannot be taken more accurately than on a mm scale. It would have been interesting if the thickness of the fragments was also measured. However, due to the flexibility of the tissue that was used, accurate measurement of the thickness would be impossible.

While analyzing the fragments obtained from chicken heart, one should keep in mind that the set-up was not able to cut complete fragments on its own. An additional knife was used to completely sever the fragment from the tissue sample. This means that the width cannot be used to judge the accuracy or precision of the set-up. However, the length of the fragments is not affected by this. When comparing the length of the fragments obtained from chicken filet with chicken heart, the same variability is seen. In Figure 20, it can be seen that the notches do not overlap. This would imply a significant difference in the median of the two datasets. However, the p-value that resulted from the two-sample t-test had a value of 0.2. This value is too high to clearly state that the two datasets differ significantly. A note should be made about the number of heart tissue fragments that are analyzed. Only nine fragments of heart tissue are measured while forty fragments of chicken filet are measured. An increase in the number of measured heart tissue fragments would increase the power of the test.

The accuracy of the experimental set-up is determined by how much the median deviates from the intended value. The intended value is the dimensions of the mouth of the suction unit. For the length, a deviation up to 6.25% is seen. The fragment width shows a deviation of 12.5%. The conclusion can be made that the set-up is successful in producing fragments with an accurate length, but the width of the fragments is larger than intended.

To determine the precision of the experimental set-up, the range that contains 50% of the samples is considered. For the length of the fragments the range is 10% and 7.5% of the median. The conclusion can therefore be made that the set-up is successful in producing precise fragment lengths. However, the width has a range of 22%. The set-up is not successful in producing fragments with a precise width.

4.3 Assessment of the proposed concept

The concept of holding tissue with suction and removing a fragment by using a knife to cut with an impulse can be compared with the current harvesting technique described in Table 1 and to the tissue slicer described in section 2.1.1. The current technique works from inside out to avoid the stiff membrane of the ovary as long as possible. The final fragments are obtained from the cortex by pushing a scalpel down on the cortex. This technique could not be translated to a minimally invasive technique because working from the inside out would result in the elimination of the complete ovary. The tissue slicer does act on the outside of the ovary. In this case, the membrane is firstly cut at the borders of the tissue slice before using a knife to cut the slice completely from the ovary. In the concept researched in this study the borders of the fragment are not cut before trying to cut the complete fragment. Although the set-up was able to produce fragments of chicken filet, it had trouble producing fragments when the tissue samples were covered with a membrane. To cut this membrane at the border of the desired fragment before removing a complete fragment could be an improvement of the proposed technique. For instance, the border of the mouth of the suction unit could be complemented with a sharp edge. This edge could then be sucked against the surface of the ovary. Further research should determine if this approach would actually cut the membrane of the ovary. A good improvement here, would be to use active suction instead of a syringe. By putting the suction on and off repetitively, the sharp edge around the suction unit would be pushed into the ovary. If this is successful in cutting the membrane of the ovary, the impulse unit could still be used to cut the fragment completely from the ovary. However, an impulse might not be necessary when the membrane is already cut. A more subtle approach could be used to cut the fragment. A knife that reciprocates would suit the pre-cut ovary better. The use of a knife that reciprocates could make the sharp edge around the mouth of the suction unit obsolete. The reciprocating knife itself could be enough to cut a fragment that is hold in the mouth of the suction unit. Therefore, the first step for further research to a harvesting technique would be to use the suction unit with active suction and a reciprocating knife to cut the fragment.

5 Conclusion

The goal of this study was to develop a technique to harvest ovarian tissue fragments with controlled dimensions in a minimally invasive way. The use of the experimental set-up showed that although it functions, the application with ovarian tissue is not viable in the current state. The experiment with chicken filet acts as a proof of principle and shows the set-up to be accurate and precise in producing fragments with a certain length. The set-up does not result in fragments with a precise or accurate width. Two important suggestions for further research are made. The first is using active suction to hold the ovary in order to obtain a higher and more constant suction force. The second suggestion is to use a different cutting technique, like a reciprocating knife, to remove a tissue fragment. The development of such a harvesting technique is important because it will improve the success rate and facilitate further research for the fertility preservation method 'autotransplantation of ovarian tissue'. This new method could improve the quality of life for many young female cancer survivors because it counteracts the loss of fertility that is often caused by cancer treatment and even allows natural conception.

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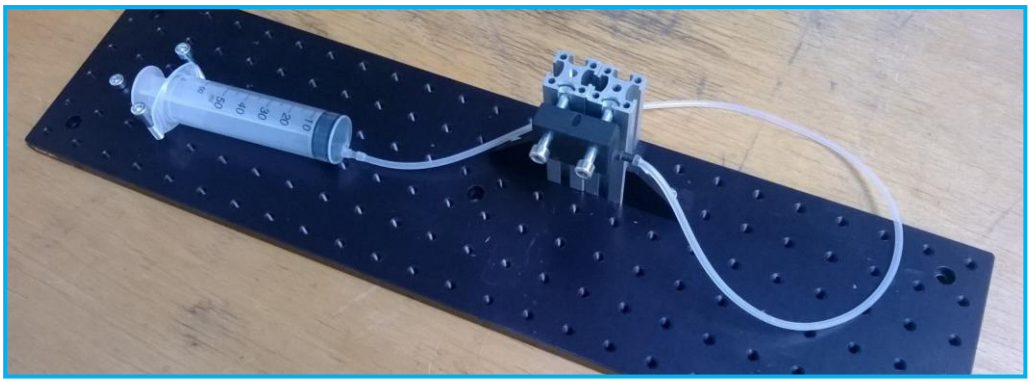
Appendices

Appendix I: Protocol suction force



Materials

- Suction force set-up
- Earring hook with ring
- Balance (Kern EMB 600-2) with a hook attached to the bottom
- Tweezers
- Tissue samples
- Meliseptol rapid
- Toothbrush

Preparation

1. Attach the earring hook to the tissue sample.
2. Weigh the tissue sample with earring hook by hanging it from the balance.

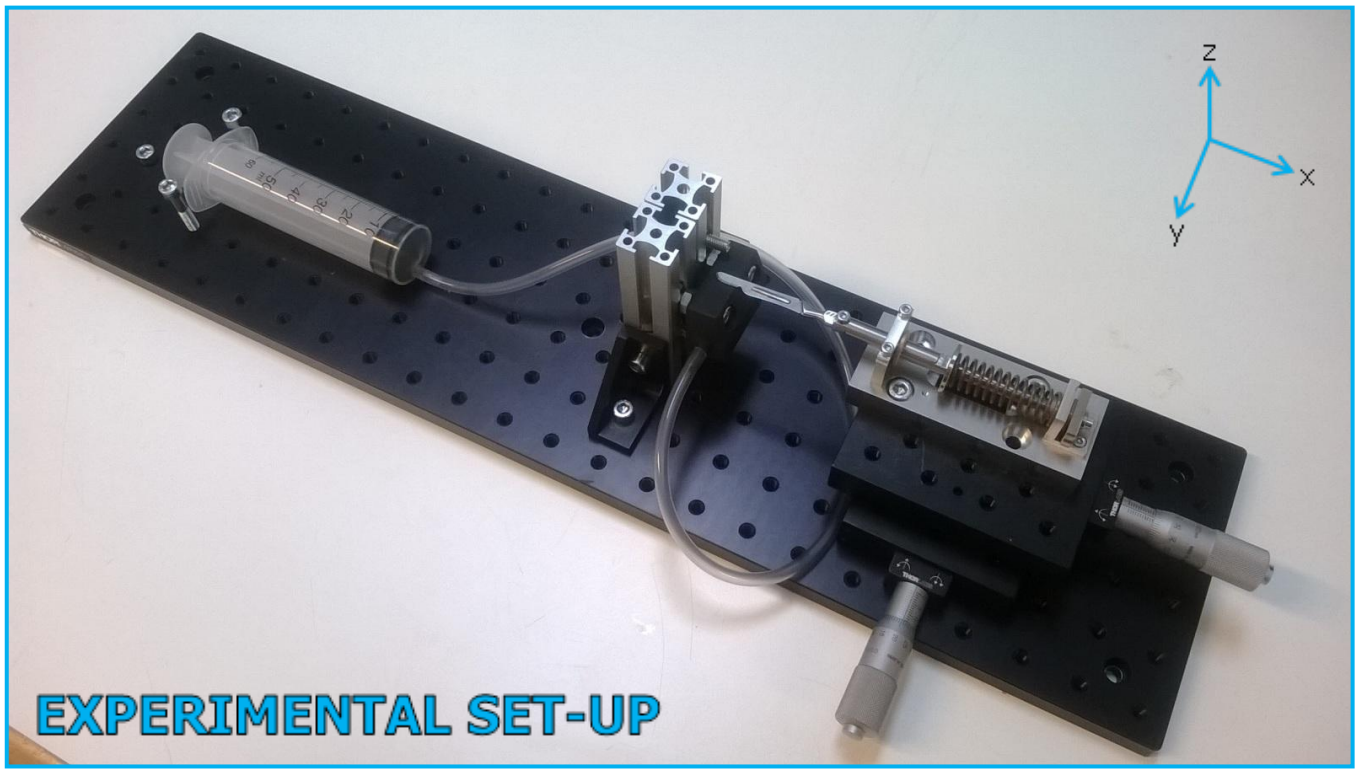
Use

3. Place the tissue sample with tweezers on top of the suction unit. Make sure the ring of the earring hook is placed in such a way the hook of the balance can grab it.
4. Apply suction with one hand
5. With the other hand move the balance over the tissue sample and catch the ring with the hook of the balance
6. Gently pull the balance upwards until the tissue sample is released from the suction unit. Meanwhile, look at the numbers on the display. The highest number resembles the force needed to pull the tissue sample from the suction unit.
7. Repeat previous steps with multiple tissue samples.

Finish

8. Clean material with Meliseptol rapid and a toothbrush

Appendix II:
Protocol experimental set-up



Materials

- Experimental set-up
- Hex key
- Tissue samples
- No. 3 scalpel blades
- Tweezers
- scalpel blade remover (e.g. Kling ex)
- Meliseptol rapid
- Toothbrush

Preparation

1. Attach a no. 3 scalpel blade to the scalpel handle of the impulse unit. BE CAREFUL!
2. Adjust the suction unit in the z-direction by tightening the two screws. Make sure the top of the suction unit is right underneath the blade of the impulse unit. WATCH OUT FOR BLADE!
3. Adjust the impulse unit in the x- and y-direction by turning the two adjuster screws of the Thorlabs Travel Translation Stages. The blade of the impulse unit should cover the mouth of the suction unit completely.

4. Attach one end of the tube to the 50ml syringe and the other end to the Luer-Slip male fitting of the suction unit.
5. Pull the handle of the impulse unit to compress the spring. Turn the handle to hook it behind the base of the impulse unit. The mouth of the suction unit is uncovered and the impulse unit is now loaded: BE CAREFUL!

Use

6. Place the tissue, with help of tweezers, on top of the mouth of the suction unit.
7. place the syringe in such a way that the sides of the barrel are in front of the two screws that are fastened to the Thorlabs Breadboard.
8. Pull the plunger of the syringe to create a suction force and hook the end of the plunger around the third screw that is fastened to the Thorlabs Breadboard.
9. Make sure no hands or other things, except the tissue sample, block the way of the blade.
10. Now turn the handle of the impulse unit back to a horizontal position to create an impulse.
11. Remove the tissue, that is now on top of the suction unit and the blade, slowly in the direction of the impulse unit.
12. Pull the handle of the impulse unit to compress the spring. Turn the handle to hook it behind the base of the impulse unit. The mouth of the suction unit with the tissue slice is uncovered and the impulse unit is now loaded: BE CAREFUL!
13. Use tweezers to pull the tissue slice from the mouth of the suction unit. If necessary, remove suction force by pushing the plunger back into the syringe.
14. Go back to 6 to cut again, or go to 15 to end or change blade

Finish

15. Turn the handle to get impulse unit back to resting position.
16. Loosen the screws of the impulse unit and remove the impulse unit. WATCH OUT FOR BLADE!
17. Remove the blade with use of a scalpel blade remover
18. Clean impulse unit and suction unit with Meliseptol rapid and a toothbrush (for suction mouth).

Note: The scalpel was changed after every four cuts.

Appendix III: Protocol fragment dimensions

Materials

- Laminated graph paper with lines every 0.5mm
- Microscope (25X – 200X USB Digital Microscope)
- Laptop with VLC media player
- Clamp to hold the microscope
- Tweezers
- Tissue fragment
- Meliseptol rapid

Preparation

1. Place the microscope above the laminated graph paper.
2. Connect microscope to the computer and focus the image.

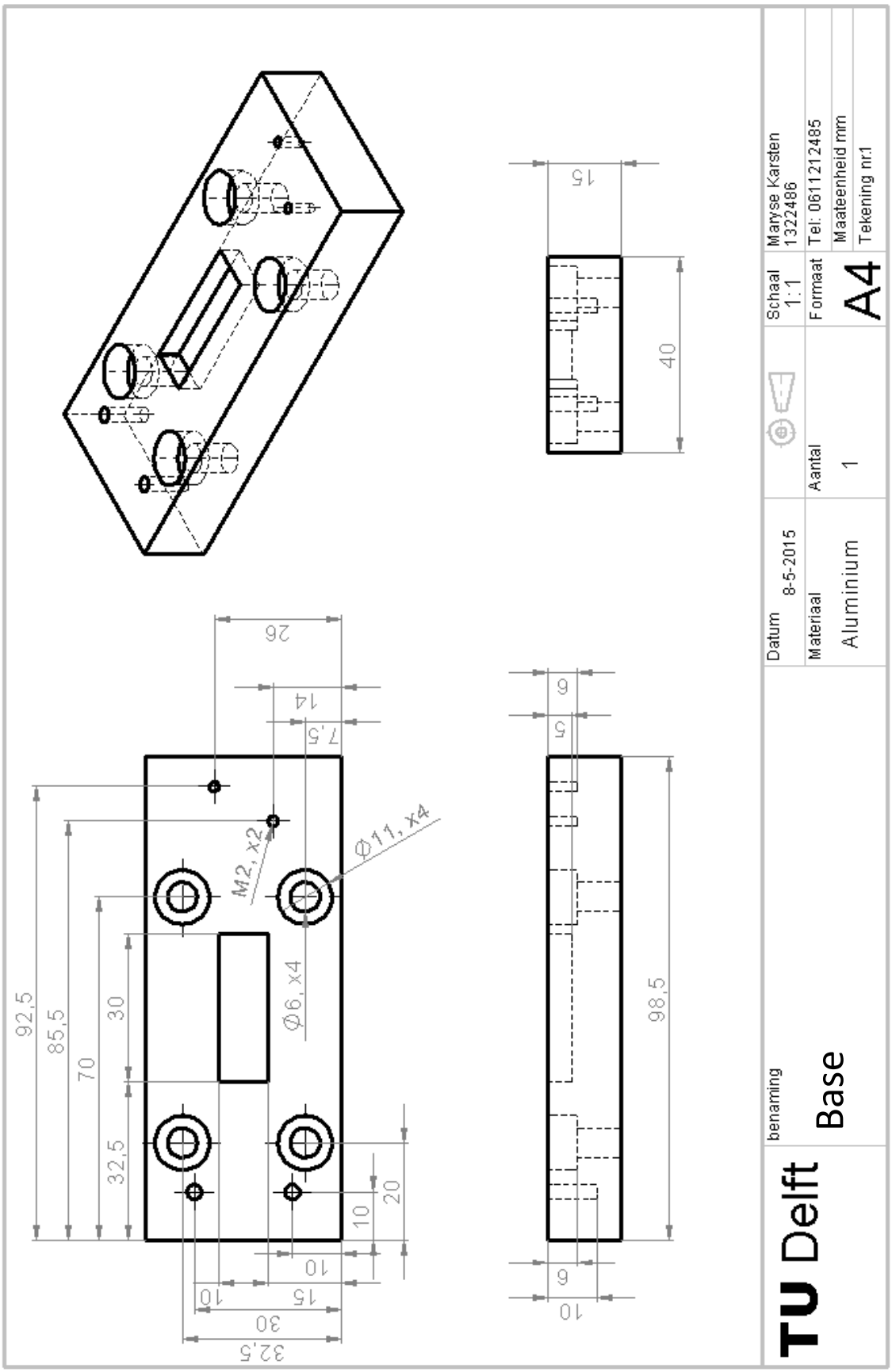
Use

3. Place tissue fragment with tweezers under the microscope in such a way that the length of the fragment is in line with the lines on the graph paper
4. Save the picture on the computer.
5. Remove fragment with tweezers
6. Repeat from step 3.

Finish

7. Clean material with Meliseptol rapid

Appendix IV: Technical drawings impulse unit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Datum 8-5-2015
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Schaal 1:1
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Maryse Karsten
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Tel: 0611212485
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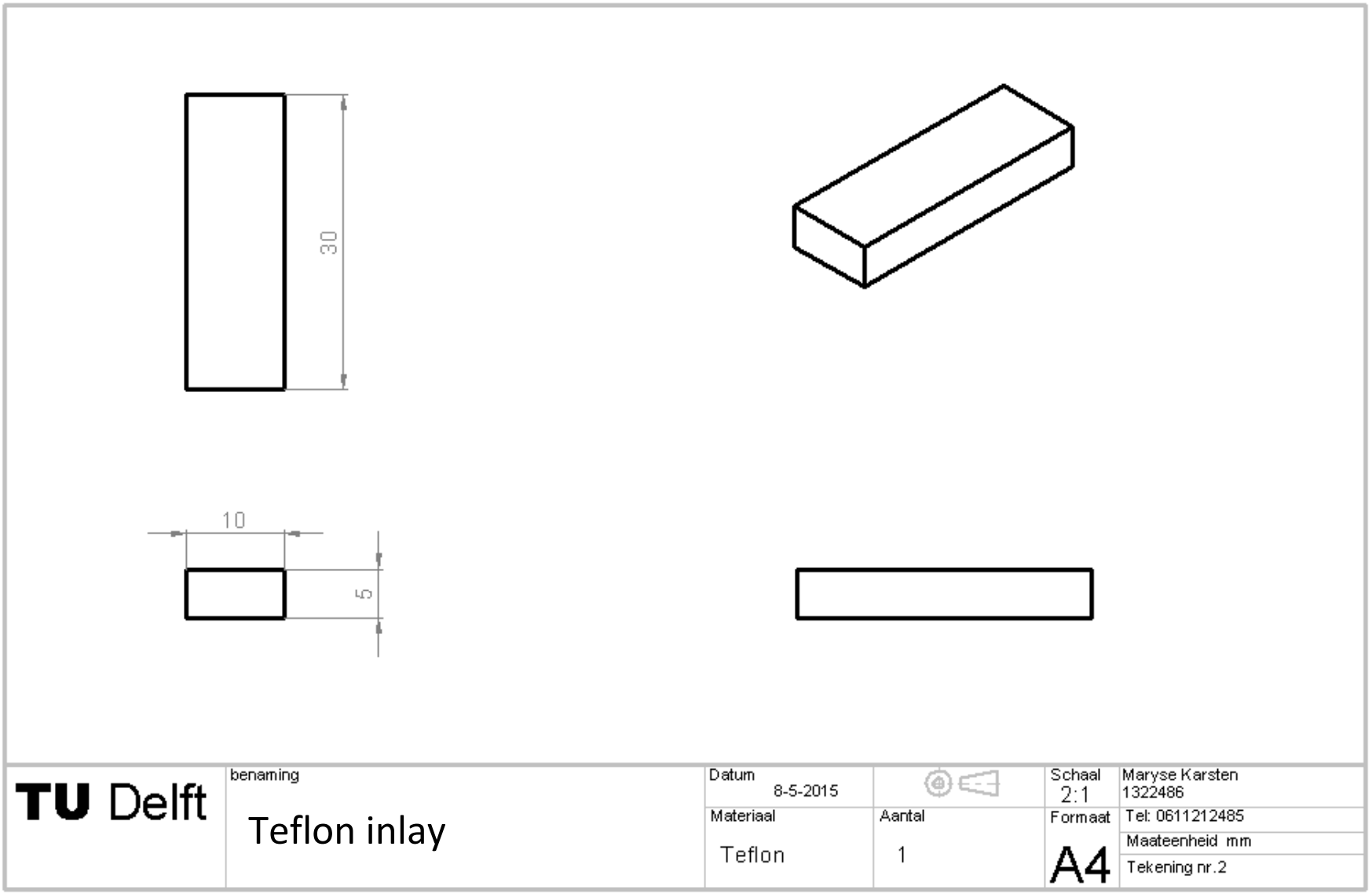

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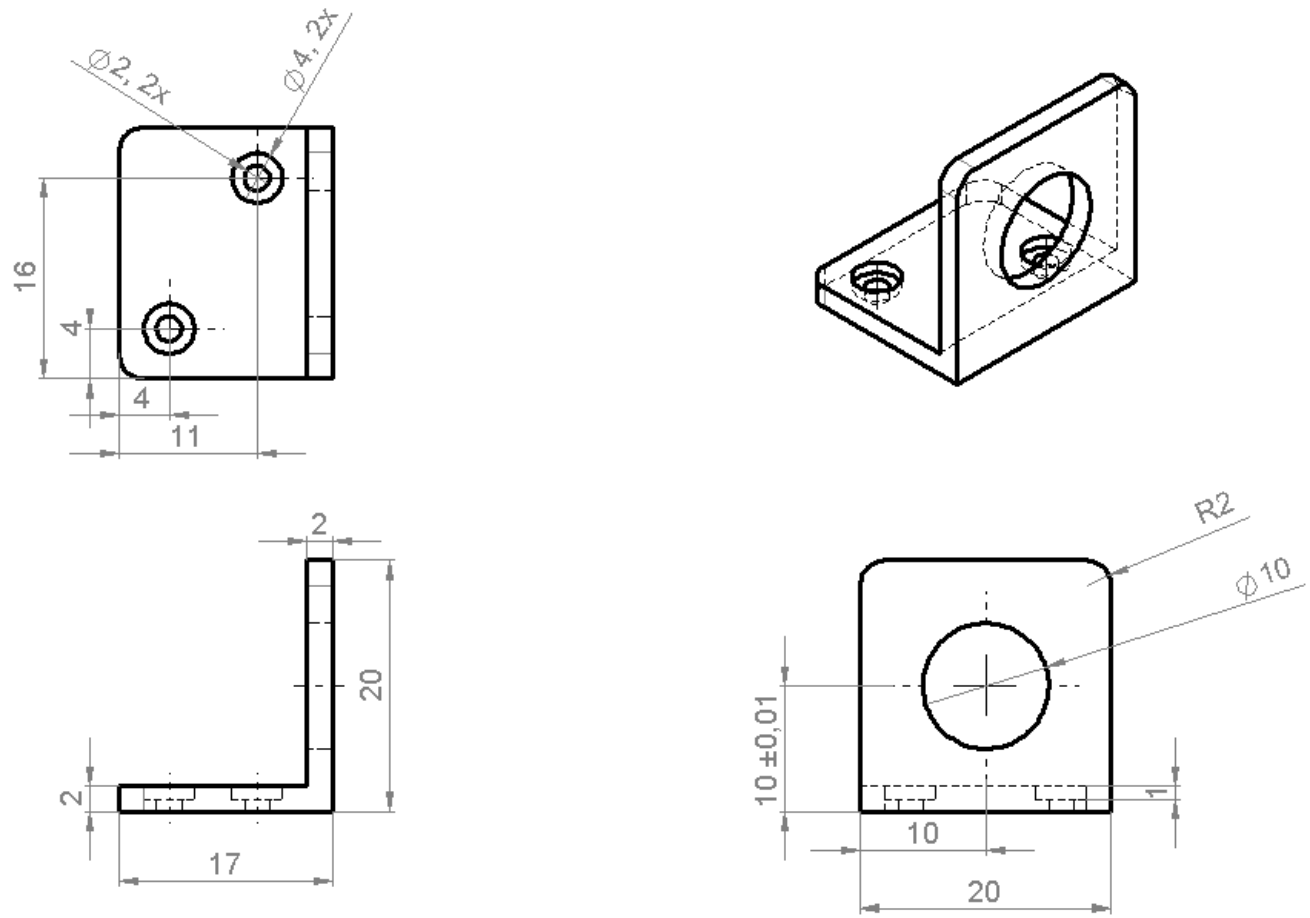

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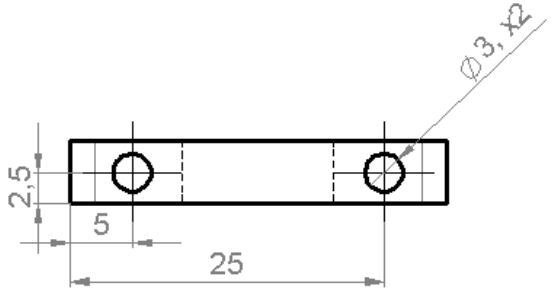

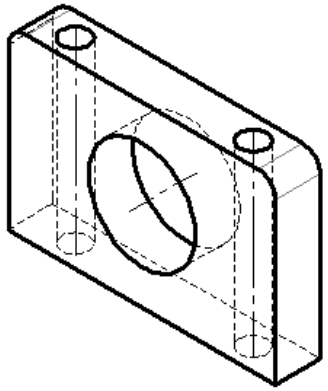

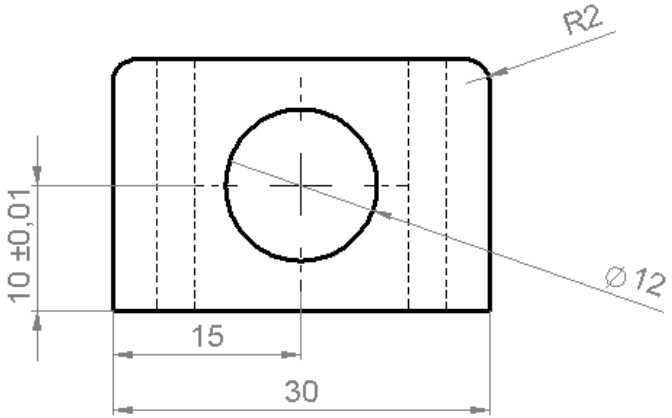

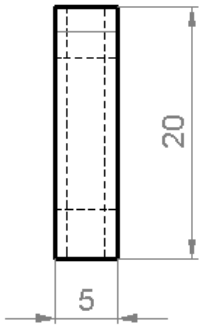

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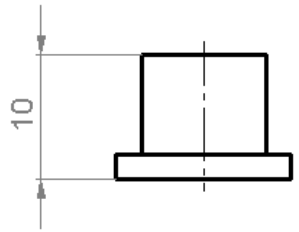

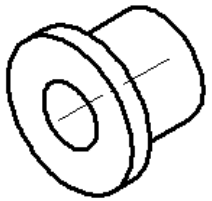

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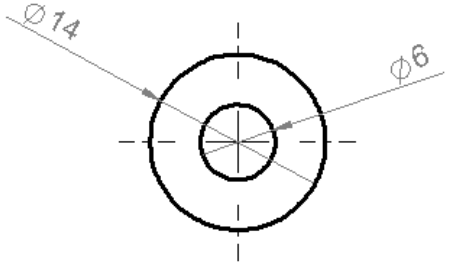

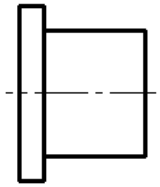

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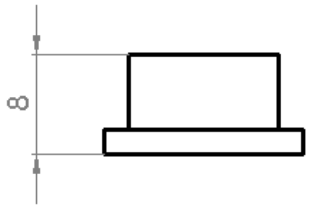

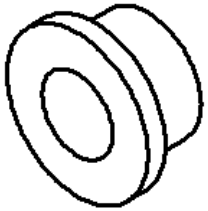

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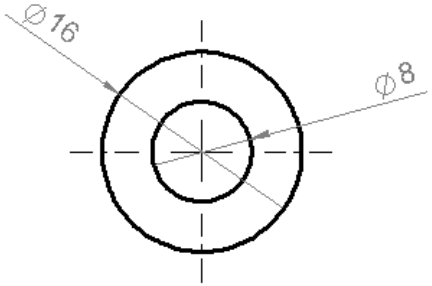

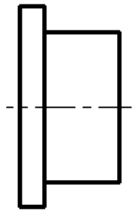

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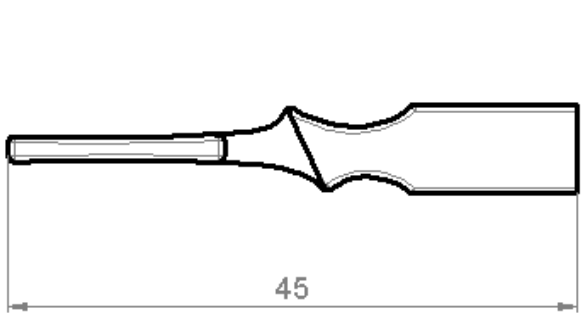

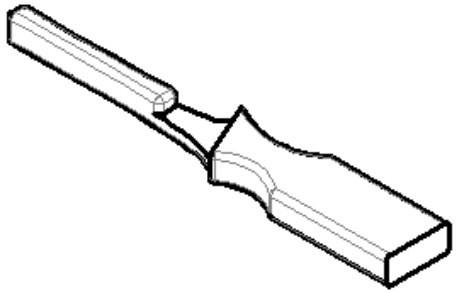

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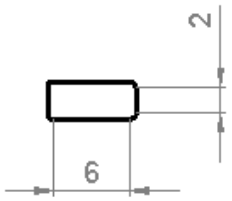

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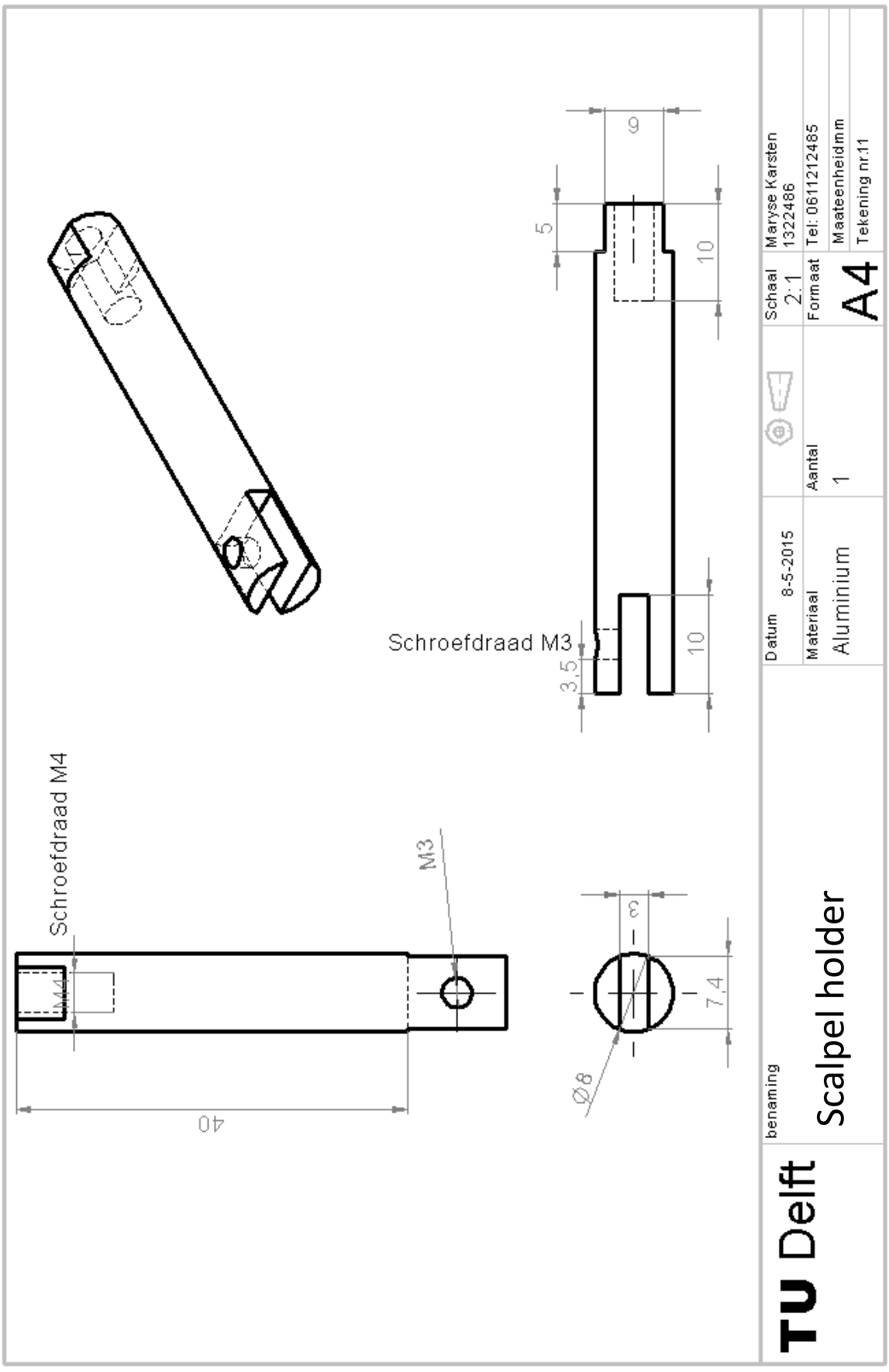
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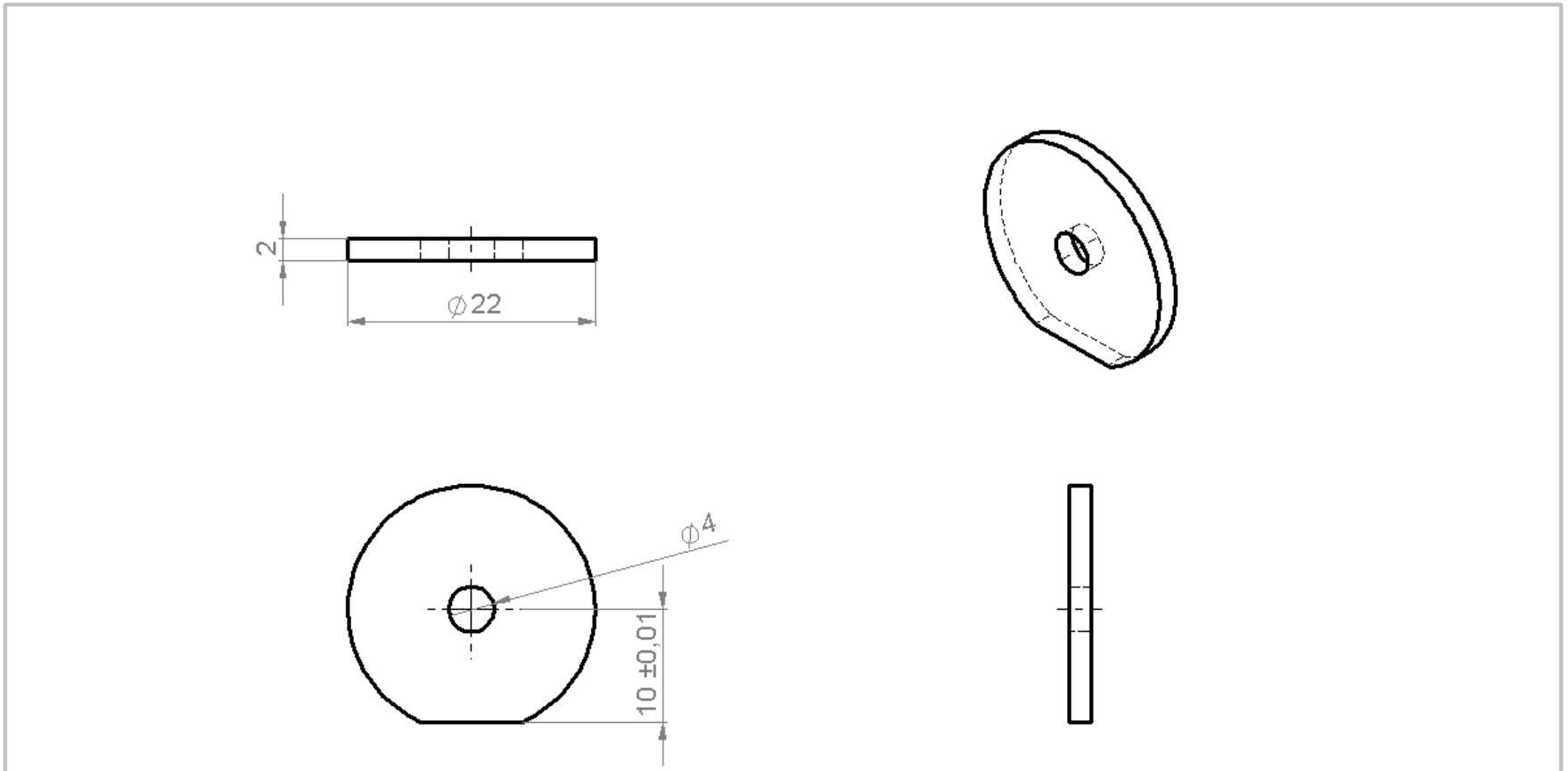

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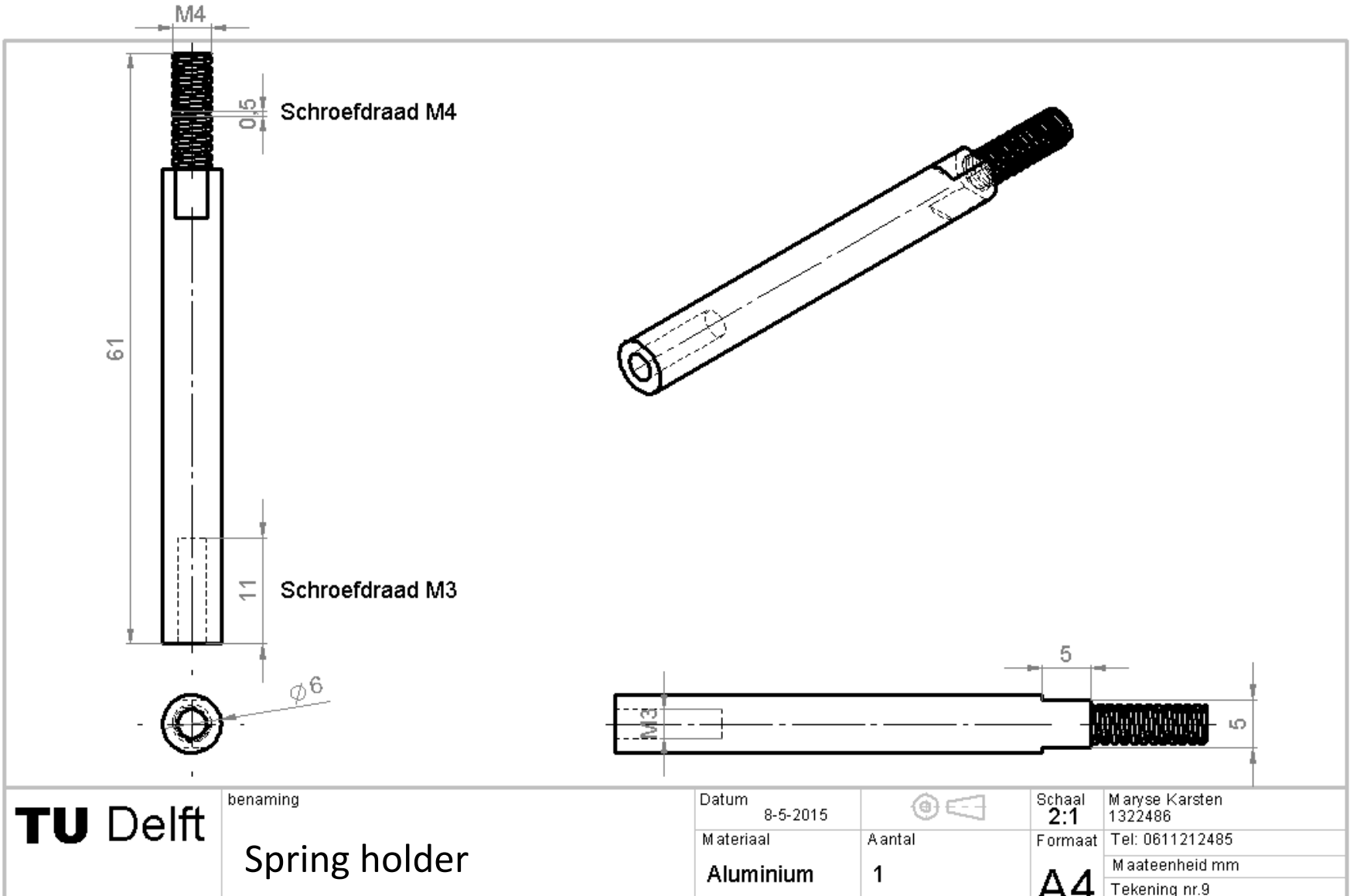

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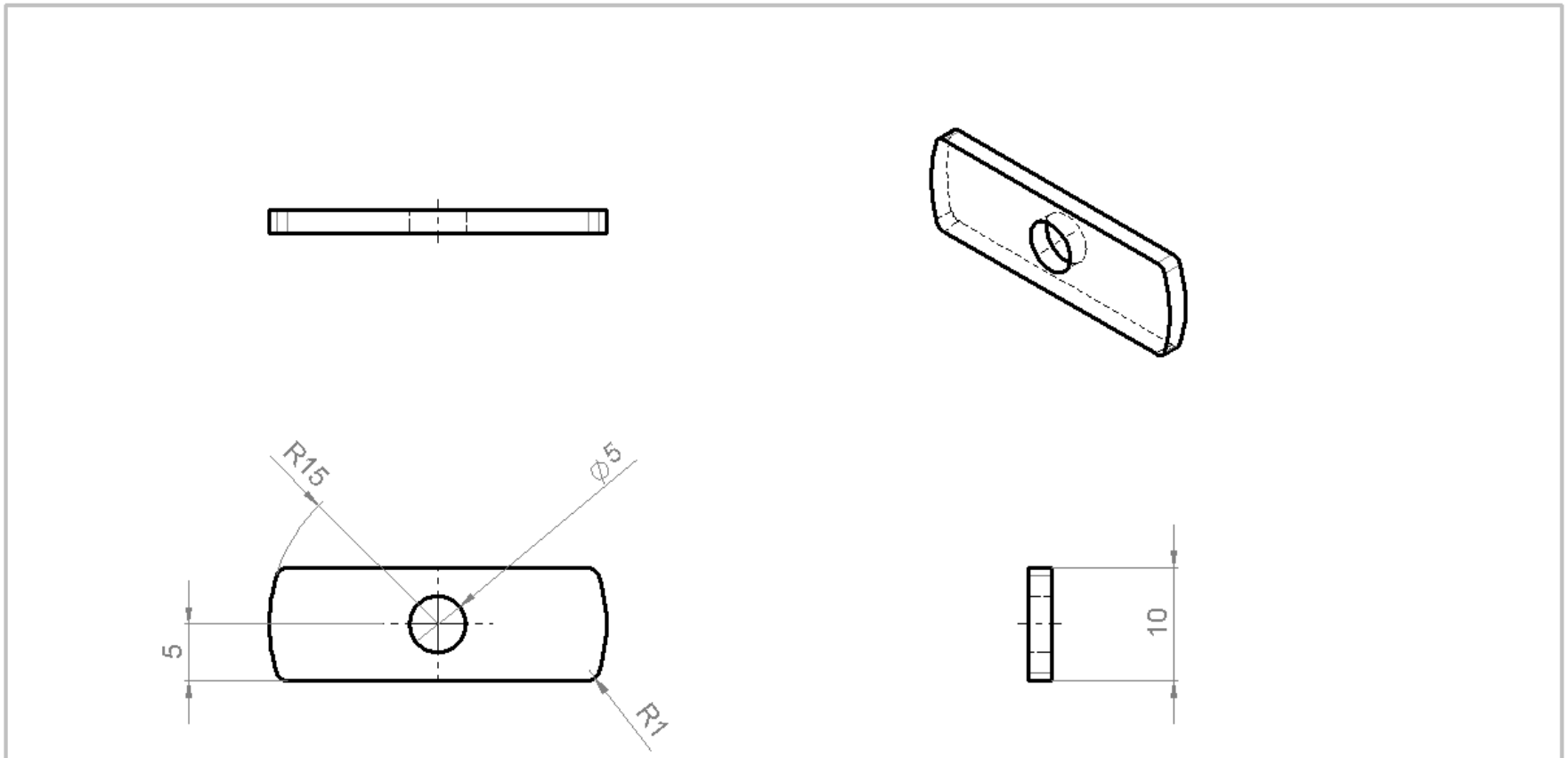
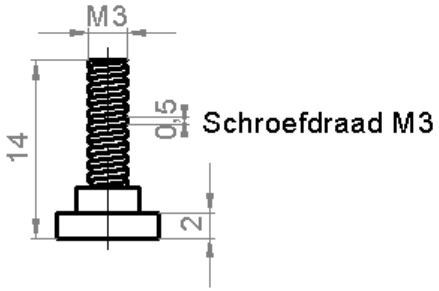
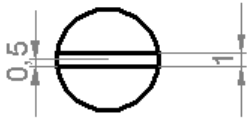
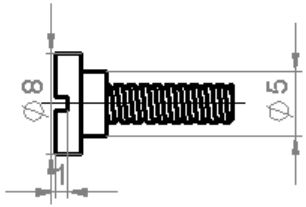


TU Delft	benaming Handle	Datum 8-5-2015		Schaal 2:1	Maryse Karsten 1322486
		Materiaal RVS	Aantal 1	Formaat A4	Tel: 0611212485 Maateenheid mm Tekening nr.8







TU Delft	benaming Handle screw	Datum 8-5-2015		Schaal 2:1	Maryse Karsten 1322486
		Materiaal Aluminium	Aantal 1	Formaat A4	Tel: 0611212485 Maateenheid mm Tekening nr.7