

TUDaThesis – Abschlussarbeiten im CD der TU Darmstadt

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Bei einer Thesis des Fachbereichs Architektur entspricht die eingereichte elektronische Fassung dem vorgestellten Modell und den vorgelegten Plänen.

Darmstadt, 10. September 2023

L. Widmayer

Acknowledgment

Ich danke euch

Abstract

Diese Kurzfassung ist kurz.

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1 Introduction

1.1 Motivation

Motivation: Biologische Proben sowohl unterm Licht- als auch Elektronenmikroskop anschauen

1.2 Task and requirements

In Application, samples (for example cells) are frozen inside a thin ice layer. The sample can be stained with fluorescence and observed with cryo light microscopy. Also a sample can be prepared to study with cryo-transmission electron microscopy (cryo-TEM). This allows to see samples in an hydrated state. This is only possible in cryo-TEM, as liquid water would evaporate in vaccum [1].

For sample preparation in cryo light microscopy and cryo-TEM, plunge-freezing is used [1] [2]. This can be done either by hand or with a plunge-freezer, but both methods use the same steps. First, the slide is held by tweezers. then a 2 to 4 ml water drop including the sample is pipetted on the hydrophilic slide. Therefore the droplet spreads over the slide. Then the water droplet is blotted with filter paper, creating a thin film of water which is evaporating quickly. Then the slide is shot in cold liquid under -140°C, for example liquid ethane. A temperature drop of over 100°C freezes the water into a thin ice layer. With this procedure, vitrified ice is formed without a crystal structure.

A vitrified sample is needed as ice crystals damage the samples and can disturb cryo light microscopy. Vitrified ice is created by freezing water abruptly into temperatures under -120°C [3]. To create a vitrified sample, the slide needs a high thermal conductivity to freeze the water quickly. Also the liquid which is used to freeze the sample should not possess the Leidenfrost effect. The vapors which are created with the leidenfrost effect will prevent a rapid temperature drop. As liquid nitrogen has the leidenfrost effect, other liquids like liquid ethane are used.

The motivation for this master thesis is to find a way to use cryo light microscopy and cryo-TEM on the same sample. But currently, no slide is found which has all requirements to be used in plunge-freezing, cryo light microscopy and cryo-TEM. in plunge-freezing, a hydrophile surface is needed to archive a thin ice layer. Additionally the thermal conductivity of the slide needs to be high for the steep temperature drop needed to create vitrified ice. For light microscopy, a transparent slide is not always required. But a good thermal conductivity is advantageous as less energy is needed to keep the sample cool (WAS FÜR VORRAUSSETZUNGEN GIBT ES DA?). In cryo-TEM, the sample needs to be extremely thin and small. Additionally, only light elements should be used as heavier electrons are disturbing the image in cryo-TEM.

To perform cryo-light microscopy and cryo-TEM, a sample transfer from one slide to another slide is proposed. The slide change must be performed at -140°C to maintain the vitrified state of the sample. Additionally

as the first slide used for plunge freezing is hydrophilic, lifting the sample is not simply possible without designing a new layer. First, I investigated lipids for potential positive characteristics for a sacrificial layer or detaching it mechanically. then I am trying PDMS and use different mixture ratios and plasma curing to make mechanical removal easier.

1.3 State-of-the-art

1.3.1 Methods for getting rid of ice xD

There are four passive anti-frosting strategies: Inhibition of ice nucleation is achieved by using surface inherent properties and heating to prevent ice crystals to form. Retardation of frosting removes water over time to prevent icing on the surface with water repellent properties such as the lotus effect. Mitigation of frost accumulation prevents already formed ice droplets to further accumulate and forming an ice layer. Last a reduction of ice adhesion so ice needs less force to detach of a surface even until ice droplets are not able to attach to a surface, detaching themselves with the force of gravity [4].

1.3.2 PDMS application (in z.b. der Flugindustrie)

PDMS is a polymer which is widely used in different applications. It is used because of its properties like hydrophobicity, biocompatibility, electric insulating. Also PDMS has low costs and allows rapid prototyping [5].

One application is the passive deicing of Aircrafts in flight. The Ice can influence the air flow around the wing, creating turbulence and reduce lift. Ice protection is therefore critical for a safe flight [6]. In this paper, PDMS is tuned for optimal characteristics in flight. To test the surfaces, flight conditions of 0.5 bar and -12°C are simulated. Fluorinated PDMS and Fluorinated PDMS is compared to aluminum, showing better resistance against ice growth. The different coatings are also examined regarding contact angle and surface roughness. Also the stability of the surface is relevant as ice can also wear down the coating itself.

2 Method

Das ist die Methode.

2.1 Phospholipids

Phospholipids are the building block of membranes in nature. They own two long hydrophobic chains and a polar head. A membrane is a bilayer of Phospholipids with the hydrophobic parts showing inwards and the hydrophilic head pointing outwards. They are also natural detergents, as they can bind to hydrophobic waste and forming an emulsion, making them removable with polar liquids like water [7].

Phospholipids are generally solvable in Alcohols(???). To apply Phospholipids, the solution is given on the surface. When the solvent dries out, the lipids are binding to the surface creating a layer. This layer can be solved again with the same solvents. If the ice layer is held by lipids, they can be used as a sacrificial layer, being solved at cryogenic temperatures. But to solve this layer, a high solubility at cryogenic temperatures must be given as the surface of the sacrificial layer is only on the edge of the sample.

2.1.1 Parylene

One idea of balancing Hydrophilic and Hydrophobic characteristics is to use Parylene. Parylene is superhydrophobic (SOURCE??? MAYBE NOT), which helps ice not to adhere to the surface. But used alone, an ice layer could not be frozen on top with plung-freezing or by hand as a water drop would not spread on the surface.

For this reason, lipids are used in combination with parylene. The lipids are holding the ice layer onto the parylene. With a solvent, the lipids can be solved and the parylene will prevent the ice layer to hold on the slide, detaching the layer. Or mechanical pulling on the ice is easier, as parylene is preventing not perfectly covered pieces from adhering on the slide.

2.1.2 Preparation lipids and cover glass

To create the slides with parylene and lipids, cover glasses (5 mm diameter) is first coated with a thin layer of parylene. Then the slides are dipped into lipid solution, covering the whole surface in lipids. Then the slides are dried, so lipids can settle on the surface.

Two different lipids are used: DOPC and EGG-PC. DOPC is stored in powder form. The first step is to solve the DOPC Powder in Ethanol (25 mg / 1 ml). Then, the solution is transferred into several small bottles. EGG-PC is shipped solved in chloroform. Two different ratios were used: 25mg / 1 ml and 10 mg / 1ml. the

pholes were broken and then also transferred into several small bottles. small bottles were chosen because if solution is coating the threads of the cap, the bottle cannot be closed airtight anymore, leading to evaporation in the flask. By splitting it into multiple flask and using the solution, only one bottle with a small part of the solution is not airtight.

2.1.3 solubility lipids

Two different solubility experiments are proposed. The first is at room temperature to find solvents which work generally at higher temperatures. With the results, first solution which don't solve the lipids can be left out of the next experiment, as there are only limited baths available. The next one is at cryogenic temperatures to find solvents which also work at cryogenic temperatures.

These tests are conducted to find a fluid to solve a sacrificial layer out of lipids.

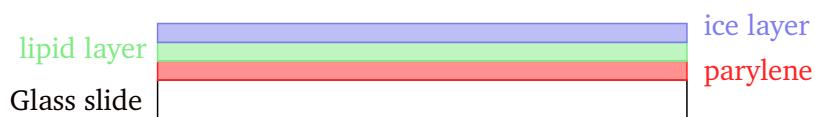


Figure 2.1: Layers of a Sample. The Lipid layer is used as a sacrificial layer. To reach the layer with a solvent, the only contact surface is to the edge. To get a fast and reliable process, a solvent with high solubility is needed.

at room temperature

First, the potential solvents are picked. For that, the tested liquids need to be safe for humans in such way that no extractor hood is needed. This is needed because the following experiment does not fit under an extractor hood. The tested substances are 4-Methyl Pentene, 3-Methyl Pentene, 1-Pentene, Isopentane, 1-Propanol, Pentane and Ethanol. Each liquid is put in a separate bottle. Then the slides are prepared as previously described.

Then a slide is placed inside each potential solvent. After 15 minutes, the slides are removed and examined under a microscope. Then the results are documented in a list. Solvent which managed to solve all lipids off the slide are now tested at cryogenic temperatures.

at cryogenic temperature

As solubility is very temperature dependent, a test is conducted at -140°C. The general process is the same, but the liquids are given in liquid nitrogen cooled baths, which are regulated to the desired temperature.

The Freezing point of tested Solutions are not all below -140°C (Table 2.1). To still test the solubility, they are partially tested at temperatures slightly above their freezing point. Also solution with a high freezing point are mixed with solution with a low freezing point as an attempt to reduce their freezing point.

In the end, the experiment has proven that solving lipids fast and reliable is not possible with tested solvents. As all solvent lipid combinations seem to be endothermic, finding a working solvent lipid combination is very unlikely.

solvent	melting point in °C
4-Methyl Pentene	-154
3-Methyl Pentene	-154
1-Pentene	-165
Isopentane	-160
1-Propanol	-126
Pentane	-129
Ethanol	-114

Table 2.1: Melting Point in °C for tested solvents.

Additionally, some solvents tested are soluble in water. It is unknown whether the solvents could be solved or diffuse inside the ice layer at -140 °C. Therefore the ice layer could be changed in some undesired manner. if a final solvent is found which is soluble in water, this needs to be addressed and tested in future experiments.

2.2 "finger"

The next tested method is detaching the ice layer mechanically. For this, a lifting assembly is used. To make this possible, the bottom layer is varied to reduce the adhesion of the ice. Also, as the assembly was not used in similar work before, different variables are addressed and examined.

2.2.1 Assemblies used at cryogenic temperatures

The assembly used to lift up samples is called finger. The finger has two main parts: A metal rod with a slightly pointed tip (Fig. 2.2). Near the tip, the rod is also temperature controlled with a temperature sensor and a heater. On the tip, a glue like HFE can be used to glue onto the sample. The second main part is a 3D printed part, containing the outer shell and routing of the cold gaseous nitrogen. The nitrogen is first flowing inside and around the metal bar for cooling. then, the Gas is flowing through an outer mantle for additional cooling and out on top.

The finger is mounted on three stages and a track. the three stages allow fine adjustment of the finger position in X,Y and Z axis. Also, when the finger is sticking to a surface, force can be applied by moving the stages in either direction. The Finger can also be moved along the rail. In use, the assembly is clamped down on the rail, to prevent additional movement. when not in use, the assembly can be moved on the rail further back. this allows access beneath the finger.

HFE (LANGES WORT) is an oil typically used as an cryoimmersion fluid [8]QUELLE ÜBERPRÜFEN!. Besides that, it has temperature dependent abilities. At freezing temperatures, it does not freeze into a solid at once. It gets more and more viscous before it freezes completely. this temperature dependency is used to first apply the HFE at higher temperatures with low viscosity and pull on the sample at low temperatures.

There are two ways of transporting samples around the work station. The shuttle fits inside the harbor. one sample can be fixed on it with screws and a brace with a hole called "window". The hole in the window exposes the upper side of the sample, allowing access for the finger and microscopy. On the other hand, small

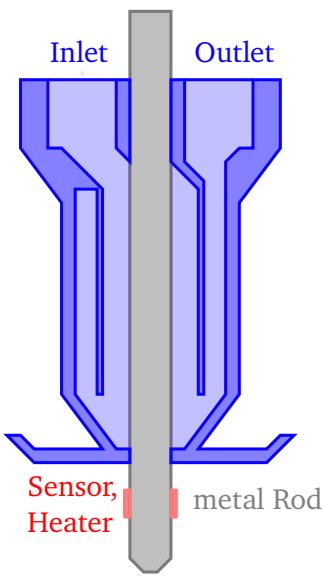


Figure 2.2: cross-section finger

container with space for three $\varnothing 5$ mm samples are used. These are 3D Printed, modified version of other containers, which can be stored in Sets of 12 in a long term storage.

FIGURE CONTAINER AND SHUTTLE.

In the beginning a smaller bath is used (Fig. 2.3). The Small bath contains two floors. The second floor is exposed and used for work. embedded in the floor are container holder, allowing transport and storage of samples. Then three elevated baths are installed for other Liquids or tools. Also a Haven for a shuttle is installed which is used for the microscopy. The small baths and the haven is elevated over the second floor, so a temperatures over -195.8°C can be regulated. The whole assembly is insulated by Nitrogen gas flowing inside the wall of the bath. Then the Nitrogen gas is blown from the brim radially to the rotation axis. The nitrogen gas separates the damp air in the room with the dry nitrogen gas inside the bath, preventing ice to form inside the bath.

This small bath can also be used with a finger. But this has some limitation: first, the space is small. The finger can be moved, but the space limits work with pincers. Additionally, the smaller baths are not needed for using the finger. also the Shuttle needs to be tilted in a specific angle so the shuttle can be moved in and out of the haven. The work flow also allows only one Shuttle in use at once, limiting throughput. Also Liquid nitrogen needs to be refilled quickly.

During this master thesis, a second bigger bath is build (Fig. 2.4). In general, the structure is similar. It also has a second elevated floor which is fabricated out of two plates screwed together. it also has indents for containers. No baths are installed, but space is held free for later addition of small baths. Also two harbors can be mounted for parellel work on two shuttles. Also both harbors can be mounted either flat or with an angle. The Bath is insulated with styrofoam and a rim with holes for warm nitrogen gas is placed on top. The holes are places along the inside of the longer side, so the stream covers the whole area with minimal turbulence. This also keeps the inside ice free.

The Shuttles are also used in cryo light microscopes. The Microscope used for cryotemperatures have an additional box installed, routing Cold nitrogen gas underneath a harbor, where the sample is placed. Heaters

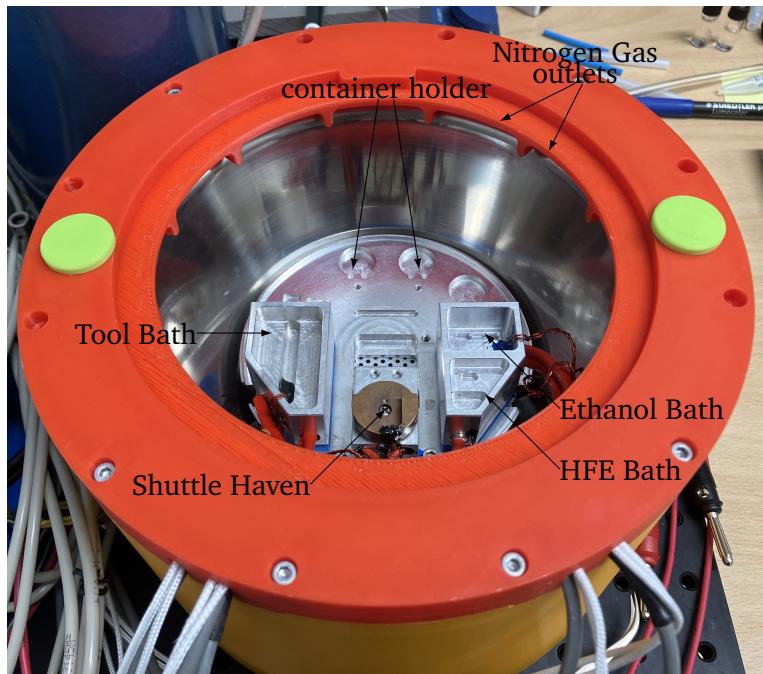


Figure 2.3: Small Bath.

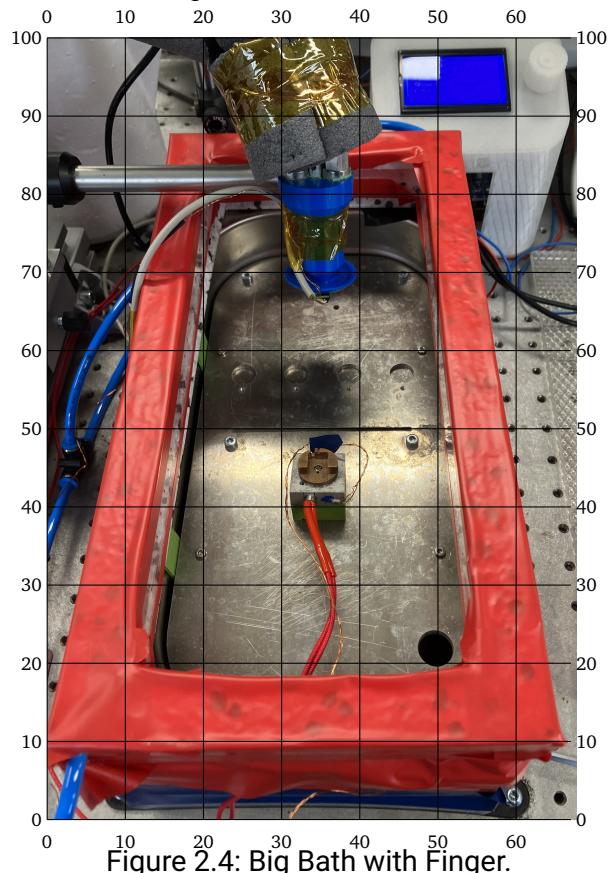


Figure 2.4: Big Bath with Finger.

are placed around the box and under the harbor to achieve a constant temperature. On top of the Harbor, warm Nitrogen is blown so no ice is forming inside the optical path.

2.2.2 general process

The goal is to mechanically lift a piece or the whole ice layer off the slide it is frozen to. The challenge is that the sample needs to stay at a vitrified state. The "finger" as previously explained is a device which can lift at a temperature range which guarantees that the sample stays vitrified.

The process has following steps: First, if not already done, cool down the finger to -140 °C. The sample is prepared and fixed onto a shuttle in the harbor underneath the finger. Then, HFE is applied on the tip. Then, the finger is positioned with the stages over the sample. After checking from different sides an camera, the finger is lowered onto the sample. Then the temperature is reduced and waited until the sample and finger is cooled down. Then the finger is pulled up by turning the stage until it detaches from the sample. After this step, if detaching was successful, the shuttle is changed to an empty shuttle. the Piece is lowered onto it and the piece is unglued by lowering the temperature. then the piece is fixed onto the shuttle.

To collect first insight, samples with parylene and lipids (as described in Section 2.1) are used. by doing those experiments, different variables where determined which could significantly influence of successful detaching. Then the different variables are examined with different experiments to improve the finger. Following variables are determined: the amount of HFE can affect the maximum force and can be falsely applied, the temperature also affects the maximum force, tensile forces and shear forces, the thickness of the ice layer, the layer between slide and ice. In the following, I will examine those in greater detail.

2.2.3 determining needed amount of glue

Using the correct amount of HFE as glue is important for a high repeatability. Too little glue is not able to connect the finger to the sample. too much glue results into a thicker layer, which is the weakest link between finger and sample. additionally, the glue can spread underneath the "window" which is holding down the sample onto the shuttle.

In first experiments, the HFE used as glue was applied with a pincer. TO archive this, the HFE is given in a cold bath at -140°C. This stops HFE from evaporating. The thickened hfe is now scooped with pincers on the tip of the finger. Though the correct amount is only determinable qualitative.

As an effort to determine the correct amount of HFE a pipette is used. The HFE is pipetted at room temperature, because at lower temperatures the viscosity is already too high. While applying the HFE onto the desired surface, around $4 \mu\text{l}$ is already evaporating. Also, sometimes the hfe does not land on the tip but on the side of the tip, where it is not very useful.

In the first experiment, I dosaged three different amount onto the tip of the finger with the pipette. The amount on the finger did not correspond to the amount of HFE. So other variabilities like the time between loading and unloading and hitting the correct spot on the finger is more relevant than the amount of HFE used.

To still determine the correct glue amount, two pictures representing the lowest and the highest usable HFE amount was picked. then the volume is calculated. this can be used as reference for future work for dosaging the right amount of HFE.

2.2.4 temperature test

Ethoxynonafluorobutane, also called HFE 7200, has a melting point of -138°C . Below, HFE gets increasingly viscous until it is hard and brittle. This property can be used as a temperature controlled glue.

In use three mode with different temperatures exist: first in "unglue" mode, the shuttle and the finger is held at -140°C . HFE has a low viscosity, which allows application on the sample. Also detachment without breaking the sample is possible. in "glue" the shuttle and the finger are cooled to -160°C , HFE hardens and force can be applied to detach the ice layer. then, "thaw" cleans the finger by heating the tip to 20°C .

As HFE is getting harder with temperature, lower temperatures in the "glue" state could allow higher forces to reliably detach the ice layer. To test this hypothesis, HFE is examined at temperatures until -170°C . (DESCRIPTON??? VIELLEICHT?)

2.2.5 tensile- vs shear mode

The direction of force is also a factor which can ease lift off of ice. Tensile mode and shear mode can be currently applied with the finger. Tensile modes advantage is the easy application with the finger. But for separating layers, this mode takes more force, as a bigger area adhering on the slide take the forces of the finger. The tensile mode would take less force, but removing a smaller part off the surface is difficult.

In application, the shuttle is tilted around 15 degrees for easier access to the shuttle. The finger is also tilted so the tip is parallel to the shuttle. To apply force, the finger can be pulled by stages in either X, Y, or Z direction. For each direction, the stress can be split into tensile and shear stress. In Z direction, mostly tensile stress is applied (Fig. 2.5 (a)). in X direction, mostly shear stress is applied (Fig. 2.5 (b)). In Y direction, only shear stress is applied, but will result in shattering of the sample, as the Ice layer is clamped down which will not allow sideways motion.

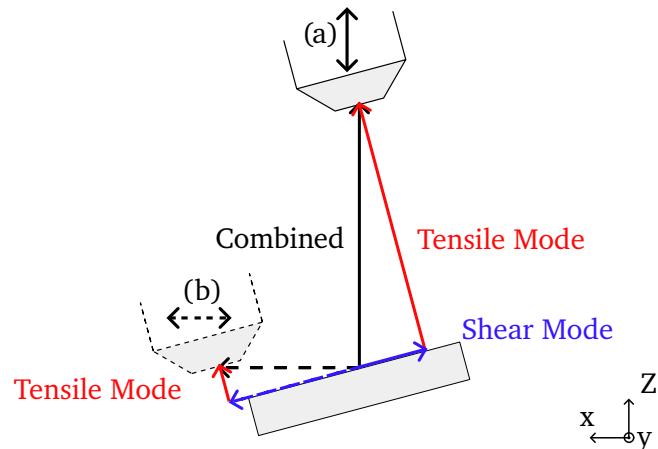


Figure 2.5: Tensile vs Shear mode

2.2.6 ice thickness and vitrified ice

To lift off a piece of ice from the ice layer, the layer must be broken in some way. Also, thicker ice sheets are harder to break than thinner ice sheets, simply of the cross section of the layer. Again, the extend of this factor

is not foreseeable.

Initially, to save time for experiments, the samples are freezed by hand in liquid nitrogen, as described before. However, the ice layers are less consistent compared to plunge freezing, resulting in mostly thicker ice layers compared to plunge freezing. also as the sample is frozen in liquid nitrogen, the leidenfrost effect is inhibiting the formation of vitrified ice.

To compare the influence of hand freezing and plunge freezing, results of lifting off samples frozen with both methods are compared. No other factors are varied in those experiments. In the end, hand freezing and plunge freezing did not make a difference. Therefore, hand freezing was also applied in future experiments, as this effect is determined as neglegtable compared to other factors.

2.3 PDMS

PDMS is as stated before a coating which is used for passive deicing. It is hydrophobic and has a low surface energy. Also it can be coat spinned into a thin layer, which is needed in this application. Also it is widely available and tunable. The only downside is that the surface needs to be hydrophile when plunge freezing. The simplest method is using a Plasma generator.

Plasma curing is also used for PDMS in Bonding (QUELLE). Also PDMS can reach under certain condition a glass like state, which makes the surface brittle and reduces the adhesion forces further (PAPER). This was done by mixture ratios of 50:1 to 100:1. Also, Plasmacuring makes the surface temporarily hydrophilic by polarizing the surface. But this is destroyed by contact of any material. Still, plasma treatment is influencing the adhesion of the ice, making it harder to remove.

Additionally, as PDMS is hydrophobic, plasmatreatment is used to make the surface more hydrophilic. Additionally, plasma treatment is changing the structure of the PDMS.

I found that plasma treatment has different effects on the PDMS on different mixture ratios.

2.3.1 Preparation of PDMS samples

I used Dowsil Sylgard 184 Silicone elastomer as PDMS [9]. It has two component, Base coat and curing agent. Depending on the Mixture ratio of these two components, the PDMS gets different properties.

All PDMS samples are prepared in a similar way only wvarying minimal in a couple of steps. The preparation starts with weighting out the needed amount of base coat and curing agent. The mixture is then stirred intensively. The mixture is placed under a vacuum bell to gas out air bubbles. Meanwhile the slides are cleaned with ethanol or isopropanol and dried. Afterwards, the PDMS mixture is coat-spinned on the slides. Then the coated cover glasses are baked in the oven.

For 1:2 base coat to curing agent weight ratio, the PDMS mixture is comparably liquid. A vacuum of 30 min is used. A coat spinning time of 120 min at 3000 RPM is results in a smooth surface for all used slides. The baking time should be at least 24 h by 80 °C. For shorter baking times, plasma treatment has a slightly different effect. Normally, touching a treated area will neutralize the effect of plasma treatment like hydrophilicity only locally on the touched surface. But here, touching the surface leads to the complete neutralization of plasma treatment when touching. In this work, the effect is undesired.

For 4:1 base coat to curing agent weight ratio, the PDMS is more viscous. The Vacuum and coat spinning are the same for 30 min vacuum and 3000 RPM for 120 min. But a baking time of 30 min at 80 °C is already sufficient to harden the PDMS.

For 50:1 base coat to curing agent weight ratio, the PDMS mixture is as viscous as the base coat. A longer vacuum of 1 h is needed to air out all bubbles. Because of the higher viscosity, the coat spinning is increased to 3500 RPM for 180 min (NACHSCHAUEN). Also a longer baking time of 20 h at 80 °C is needed to harden the PDMS.

Additionally, rectangular cover glass with 20x20 and 24x40 are coat spinned and split in multiple smaller pieces to speed up the sample preparation process. The process is the same described as before for each PDMS mixture ratio, but with two additional steps.

Before coat spinning, the glass is scratched with a diamond Pencil. This helps with breaking the glass into smaller parts into predefined parts. Then, tape is put over the scratched glass. Then the pdms is coat spinned onto the glass with tape on the bottom side.

After baking in the oven, the glass the glass is broken into smaller pieces. Then the glass is fixed again tape side down to the table and is covered with PLASTIC (WIE HEI?T DIE FOLIE???). Then smaller sample pieces can be broken off by loosening the broken glass pieces with flat pincers.

This method has advantages as well as drawbacks to coat spinning small glass pieces separately. First, time is won by coat spinning, as one big glass can be split into several smaller ones. But with hand scratching, only irregular and rectangular shapes can be won out of the bigger glass piece. also at breaking and loosening with pincers, samples are lost. This is a result of cracks forming through rough handling with the pincers and cracks also forming at non scratched parts.

2.3.2 Influence of plasma treatment on PDMS

As not much data is known about Plasma treatment on certain PDMS mixture ratios,

In one paper, the influence of plasma treatment on Mixture ratios of 50:1 to 100:1 is described [10]. A thin layer of those pdms mixtures is put onto a preformed PDMS piece with lower mixture ratio. The preformed piece is used to apply shear stress to the surface by stretching the lower PDMS form piece. Therefore only tensile force is examined in this paper. It shows no significant difference between described mixture ratios. It also shows that higher plasma treatment leads to more brittle surfaces, reducing the force needed to break the PDMS Layer by 90 %.

Also known is the adhesion force on PDMS on different mixtures from 1:3 to 50:1 mixture ratio [11]. A block of PDMS is molded with one mixture ratio. Then, an Ice block is frozen on top of the PDMS. Hooks are attached on both sides and a pulling machine is pulling on the ice and PDMS. This is done with tensile and shear mode. It shows that mixture ratios 10:1 to 1:3 have significant lower adhesion forces on ice. At for example at 2:1, the shear mode is just under 20 kPa and the tensile mode around 30 kPa.

Still, the effect of plasmaactivation under 50:1 is unknown. The Plasmaactivation could have two effects: first, it can increase the adhesion on ice to pdms. second, the pdms could change it structure, which could increase as well as decrease the strength of the PDMS layer. In the following, the effect of plasma activation on PDMS is examined.

setup

To test the tensile strength of different PDMS mixtures and the effect of plasmacuring, a Pulling machine (NAME RAUSFINDEN) is used. On the Top part, two sensors are installed. The upper Sensor is rated for 2 kN (ÜBERPRÜFEN). the lower sensor is rated for up to 100 N. As the upper one is extremely stiff and the forces are \ll 2 kN, the upper Sensor can be assumed as inflexible. On both the upper and lower part, two clamps are fixed onto the machine. On the Bottom Clamp a 3D-Printed stage is used with threaded holes for screws.

Glass slides with coated PDMS are used, which are fixed on the bottom end of the machine (Fig. 2.6). On the top end, waterjet cut stams are clamped on. Then a drop of uv glue is put on the top part. the top part is lowered onto the bottom side. Then the uv glue is cured with an uv pistol for 3 minutes (1.5 minutes from the left 1.5 minutes from the right). Then the sample is pulled with constant distance.

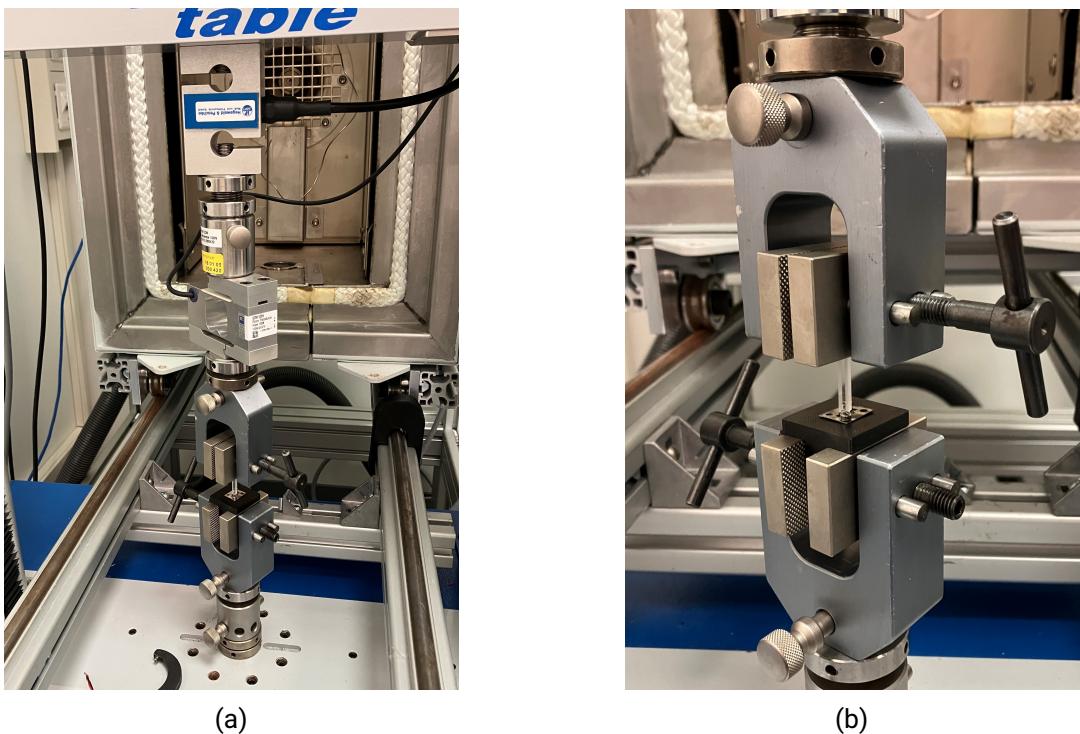


Figure 2.6: Setup on the Pulling Machine.

Because Glue dosaging varied a good amount, the Area is not assumed to be the bottom of the stamp. After Pulling, the glue is sticking on the stamp, outlining the area of the glue. The glue is analyzed under a microscope and the area is measured. With the Area and the maximum Force shortly before detaching, the Tensile stress is calculated. Then the experiment is repeated.

2.3.3 detaching ice from PDMS

1:2 and 4:1

3 Results

3.1 Lipids

In the previous chapter, the method of using a sacrificial layer to detach Ice was discussed. For this, lipids need to be solved at cryogenic temperatures. As not every lipid is solvable the same way in different solvents, a first test is conducted to obtain the potential solvents at room temperature. Then the best solvents are also tested at cryogenic temperatures.

The solubility of lipids at room temperature in different solvents are tested. For this experiment the cover glasses are coated with lipids. Then a first reference image was taken. Then the cover glass is given into a small container with the potential solvent. After 15 minutes, the cover glass is removed and compared under the microscope with the reference picture. If streaks created from lipids are still as visible as before, the lipids are categorized as insoluble in this solvent. If the streaks partially disappeared and/or are less visible, the lipids are categorized as partially soluble in this solvent. Last if the streaks completely disappear, the lipids are assigned as soluble in the solvent (Table 3.1).

potential solvent	solubility EGG-PC	solubility DOPC
4-Methyl Pentene	soluble	N/A
3-Methyl Pentene	slightly soluble	insoluble
1-Pentene	insoluble	insoluble
Isopentane	soluble	slightly soluble
1-Propanol	soluble	soluble
Pentane	soluble	insoluble
Ethanol	N/A	soluble

Table 3.1: result of solubility tests at room temperature. soluble indicates solvents which are able to visibly solve all lipids off a cover glass. slightly soluble indicates solutions which are able to solve lipids, but some stains are left: insoluble indicates no visible changes of tested lipid.

This experiment shows that three different solvents exist for each EGG-PC as well as DOPC with high solubility (Table 3.1). Following those results, solvents categorized with "soluble" are tested regarding solubility at temperatures of -140°C . As not all solutions are liquid at -140°C (Table 2.1), they are tested at higher temperatures above their melting point, as mentioned in chapter 2.1.3. In addition they are tested as mixtures with other solvents with a lower melting point, to lower its melting point. Additionally all lipids are tested in liquid ethane. Ethane was not tested at room temperature, as the boiling point is at -88.6°C (ZITAT PUBCHEM ETHANE).

This experiment shows that no tested solvent was able to completely solve lipids at -140°C and within 15 min (Table 3.2). Also the smears of lipids did not only stay partially behind, but also new streaks appear on the glass slides. This means that some lipids redistributed on the glass slide.

Using solvents to destroy a sacrificial layer, a high solubility is a requirement. In this case, the sacrificial layer would be completely covered by the ice layer except the edges. So the solvents have only a small area to start solving the layer. To solve it completely, a strong solvent is needed to detach the ice layer from the slide. Additionally, as the ice layer needs to stay vitrified, the temperature cannot be raised over -140°C .

The solving process of lipids in solutions is probably endothermic. This means that heat is needed to solve lipids, so cold temperature heavily decrease solubility QUELLE DENNIS ODER SO. This effect was observed over the last experiments by all solvents to varying degree. It can be assumed that the majority of solvent lipids mixtures are endothermic which is very disadvantageous for finding a potential solvent lipid candidate. Strongly exothermic solvents could heat up the ice enough to create ice crystals, which would not be feasible. So only weakly exothermic solvents are feasible for this task.

Solvent	Result
Pentane	soluble at -125°C
4-methyl pentene	insoluble
1:1 volume ratio HFE to 1-Propanol	did not mix, slightly soluble
Liquid ethane	insoluble

(a) EGG-PC

Solvent	Result
1:4 volume ratio 1:2 molar ratio Ethanol to Isopentane	slightly soluble
1:2 volume ratio 1:1 molar ratio 1-Propanol to Isopentane	insoluble
Isopentane	slightly soluble
1-Propanol	at -130°C slightly soluble
Liquid ethane	insoluble

(b) DOPC

Table 3.2: in 3.2a for EGG-PC, no sufficient solubility at -140°C was found. In 3.2a, DOPC was tested but also no proper solution was found.

As finding a good solvent lipid combinations seems very unlikely, a new method was tested. In the next section, the finger tool is used to try mechanically detach the ice layer.

3.2 Finger

For this section, cover glass coated in Parylene are used as object slide. The slide is then dipped in solution containing lipids for a lipid coating. A ice layer with fluoriscine is frozen with either plunge-freezing or using

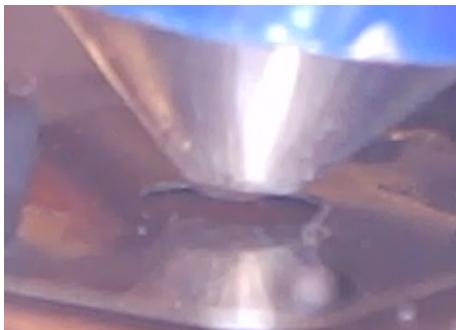
a pincer and liquid nitrogen. Additionally, the "finger" is used as tool to try lifting off a piece of ice from the frozen layer on top of the lipids. In the next sections, different variables are examined and tested.

3.2.1 Finding right dosage of HFE

First obvious variable and potential issue source is the amount of HFE used as glue. High dosages of liquid HFE can spread underneath the frame holding the sample, leading to an inefficient force distribution. Also a big glue layer is a weak point between finger and sample, leading to a reduction of maximum force which can be applied. Too little glue will not connect the finger to the sample. Additionally, the dosaging of glue revealed to be a big challenge.

The HFE is dosaged with a pipette. The HFE is "taken up WORD" at room temperature, then the HFE is "released WORD" on the tip of the finger. In between, HFE is evaporating. Around $4\ \mu\text{l}$ is evaporating each time. Based on this knowledge, dosaging $4.10\ \mu\text{l}$, $4.30\ \mu\text{l}$ and $4.50\ \mu\text{l}$ is compared and a picture is made.

Results show that pipetting HFE is not reliable. The range spreads of too little to too much HFE even for those dosages. Not only differences in evaporation are playing a role. Correct placement on the tip is a major factor of glue dosaging. Still, a visual estimate for the correct glue dosage can be made by calculating the drop volume out of camera images.



(a) chosen example for lower limit



(b) chosen example upper limit

Figure 3.1: example of upper limit and lower limit for glue dosages. (BETTER PICTURES NEEDED)

To calculate the actual glue dosage, two exemplary Pictures of an Upper and lower limit of glue dosages is picked. Then the Volume is calculated with a formula for the volume of a spherical section. All needed components are calculated out of the estimated contact angle of the glue $\alpha \approx 45^\circ$ and the tip diameter of $d = 1.68\ \text{mm}$, for the lower range a reduction of d by a factor of $\frac{2}{3}$ is assumed as the drop is not covering the whole tip. The resulting volume range of the glue dosage is $0.11\ \mu\text{l} \gtrsim V \gtrsim 0.38\ \mu\text{l}$. Also the lower end of this range is desired, but the repetition range in correctly dosaging lower doses is lower.

3.2.2 Temperature over applied force

As the HFE gluing effect is temperature dependent, the temperature needs to be regulated precisely. To narrow in the temperature dependency of HFE, Different temperatures are tested. Also at some point, cracks form in HFE. Those cracks make HFE brittle so less stress can be put on HFE without breaking.

(SKIZZE?)

In application tests on lipid samples, the temperatures -150°C, -155°C, -160°C, -165°C and -170°C are compared. A needle is put in the HFE to subjectively observe the mechanical properties of HFE at certain temperatures.

At -150 °C, the HFE is still only lightly viscous and cannot hold up the needle. Reducing the Temperature to -155 °C results in more viscosity, but still not enough to hold up the needle. At -160 °C the HFE is viscous enough so that the Needle is held up by the HFE. Also the Needle can be pulled out and the HFE is closing the gap. Also with enough force, the Needle can penetrate the HFE. Also at -165 °C, the HFE gets more viscous, the Needle is harder to pull out or put in the HFE. also no cracks formed so far. Now at -170 °C, It hardens further, The HFE is still viscous, but with wiggling, the Needle can now be pulled out easier. Also if the Temperature is only a bit under -170 °C. Cracks form and the Needle is easily removable but penetration is impossible.

Heating the cracked HFE up results in cracks eventually disappearing. at -165 °C, first cracks disappear, but a lot remain, which is still lowering the mechanical stability of the HFE. With -160 °C still some cracks remain. Heating the HFE to -140 °C will result in cracks completely disappearing.

At -165 °C, maximum stress load can be applied. Higher temperatures have lower viscosity, which lowers the maximum stress before HFE breaks. At -170 °C, if the temperature not precisely regulated, eventually cracks will form, lowering the maximum stress. Also HFE is the weakest link between Finger and Shuttle. So maximizing the HFE stability directly results in higher forces which can be applied onto the ice layer.

To test how applicable the temperatures are, pulling test are done as described in section 3.2 except the temperatures of the finger is lowered to -165 °C and -170 °C. With extra attention to proper insulation and no leakage of the cold nitrogen gas, -165 °C is reachable and can be held over time. Still, it is not practical as leaks are sometimes spotted late. Then parts of the pipes need to be heated up, disconnected and then properly reconnected and then cooled down again. -170 °C cannot be reached and held by the current finger.

Therefore, the finger can be used at -165 °C. But for better reliability, smaller improvements should be made to improve reliability of the finger. Also if the Sample is cooled down too much accidentally, the setup should be heated up to -140 °C to iron out cracks which potentially formed at the reduced temperature.

3.2.3 Tensile mode vs Shear mode

As mentioned before, Force can be applied by moving the stage in either X, Y or Z axis. In the tilted position of the harbor, moving in Z axis splits in mostly tensile mode but also shear mode. This is used in almost all experiments.

In a couple experiments, moving in X axis is tried instead. Generally, way less force is transferred. this is feelable on the stage and hearable at detachment. The reason is probably the HFE not being able to handle high shear forces. (kann ich das beweisen???)

3.2.4 Detaching ice with finger of plunge freezed samples

Next observed possible factor is the thickness of the ice layer. In the following, samples freezed with a plunge-freezer are compared to samples freezed with a pincer in liquid nitrogen. The results are categorized in 4 categories: Not successful pulls don't have visible changes of the florescent ice layer, Partially successes are visible breaks or clear movement of ice parts on the ice layer, Successful liftoff is a missing piece and a visible piece on the finger, which could be used for future steps. In the results, there is no difference between

Category	Hand-freezed	Plunge-freezed
count executed tries	4	4
unsuccessful	3	3
breaks/movement of ice	1	1
piece lifted with finger	0	0

Table 3.3: Comparison of detachability between hand-freezed and plunge-freezed samples

Hand freezed and plunge-freezed samples regarding detachability. Therefore Ice thickness is not a factor which makes detaching ice easier. As both methods don't show success in detaching ice pieces, it could still be a relevant factor but not a thing which should make a certain solution magically work xD

3.2.5 other observed error sources??

During experiments, other error sources are found.

Wrong positioning, forming of ice

The formation of Ice inside the bath is largely inhibited through the Nitrogen Gas inlets. The nitrogen is forming a barrier to the atmosphere which contains humidity that turns into ice at cold temperatures. However, through turbulence and the finger, some ice can form inside the bath.

In some experiments, high buildup of additional ice is observed on the sample. The additional ice is looser, reducing the possible grip onto the desired ice layer. The formation is traced back to a leakage in the finger. The tolerances between 3D printed part and metal bar should be able to seal the gap airtight. But through changing out parts, the tolerances are looser, allowing a weak cold nitrogen current right on the sample. There are two possibility where the humidity itself is coming from: The cold nitrogen gas itself could contain some humidity. Second through additional turbulence, more air is sucked through the barrier. The air is directed directly on the sample, causing more ice buildup. To avoid this, the gap is sealed tight with tooth silicone????

3.3 PDMS

To speed up the process of finding the right balance of PDMS Mixture ratio and plasma curing, experiments at room temperature done. For this, a pulling machine is used. First the sample is prepared. Then, the sample is clamped to the pulling machine. Then a plexiglass stamp is aligned on top of the sample. With UV glue, the stamp is glued to the PDMS layer on the sample. Before gluing, the Force and distance is set to zero on the pulling machine. After gluing, a couple minutes are waited so no further stress changes are ongoing from the gluing process. then the Machine is pulling on the sample with constant velocity. After detachment, the measurement is stopped. Afterwards, the stamp and the layer are analyzed under a microscope. The area is determined. With the maximum force and area, the maximum stress is calculated. Each experiment is repeated multiple times.

3.3.1 experiments at room temperature

In between experiments, small variations are made: two different stamps are used, one has an area of 2 mm x 3 mm and another stamp is 3 mm x 3 mm. Since the area is measured afterwards, this should not have a significant effect on the results. Also, in the beginning, while waiting of the stress changes to subside, the pulling machine was inactive. with this, the Force before pulling will be higher as zero. Before pulling, the machine is set back to zero. After pulling, there is an offset between the neutral value and the value before because of zeroing, so the offset needs to be corrected. To avoid this, the pulling machine is set to zero force while waiting instead. Then no offset correction is needed. The offset correction and new method increased the accuracy between pull tests.

To verify the setup, samples coated with 4:1 and 1:2 curing agent to base coat weight ratio and uncoated coverglass used as slides are compared. The results show 2:1 mixture ratio with 87.3 ± 19.9 kPa is easier to detach than 1:4 mixture ratio with 429.1 ± 5.1 kPa (Fig. 3.2). Also glass without PDMS takes up a lot more tensile stress with 1161.5 ± 111.5 kPa. sometimes the machine is able to break the glass. Under the microscope, it is not visible if the PDMS layer itself was lifted from the glass or not.

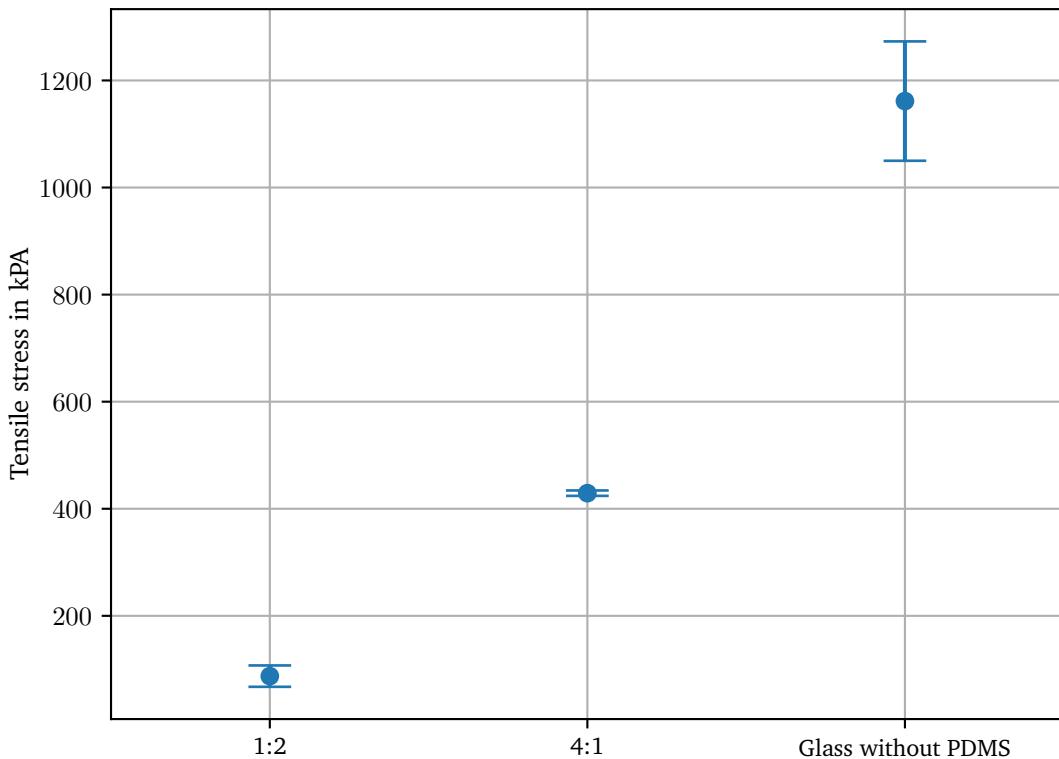


Figure 3.2: Comparison 4:1, 1:2 Base coat to curing Agent and glass without PDMS

In literature, the ice adhesion on PDMS without plasma treatment is 35 kPa. for 2:1 and 5:1 the stress is between 60 to 80 kPa [11]. This is lower considerably lower than the experiment before. Therefore, one limitation is that the actual adhesion between ice and PDMS cannot be simulated by this experiment. Still,

there is a correlation between the values and the experiment can give an insight of PDMS durability. In the end, if the separation happens between ice and pdms or pdms and glass are both good results.

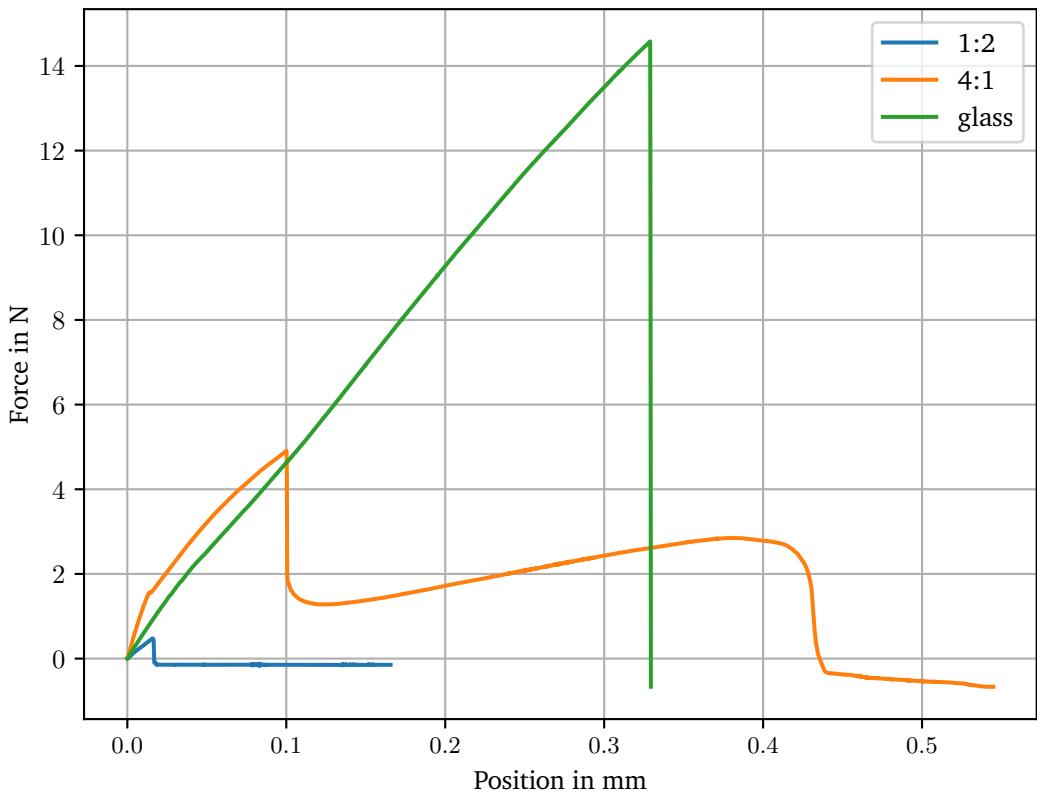


Figure 3.3: force over Time

In the next experiment, the effect of plasma curing is investigated. As the mixture ratio of 1:2 has the lowest adhesion, this experiment used this pdms mixture ratio. The same setup is used. Samples with a 2:1 weight ratio PDMS are additionally plasma treated before quickly clamping on the pulling machine. Even with low repetition rates, a clear tendency can be observed. With lower and stronger plasma treatment, the durable the PDMS Layer gets (Fig. 3.4). Over the whole range, The needed stress sextables. Because the repititon rate is low, the exact values should be treated cautiosly. Also the results are not applicable to other mixture ratios, as different behaviour in plasma activation was observed between 2:1 and 4:1 weight ratio. also no glass-like state was observed in 2:1 weight ratio mixture.

As PDMS is hydrophobic, plasma activation is needed to freeze a thin layer of ice onto the coated slide. still, the lowest setting in the plasma machine(???) is not neccessarily enough plasma activation to get a low enough plasma angle. In a small test, PDMS coated slides with mixture ratio 1:2 is plasmaactivated with 25% for 0.1 min, 30% for 0.2 min and 35% or 0.3 min. 25% for 0.1 min does not deliver a low enough contact angle. 25% for 0.2 min contact angle is already very low. 35% with 0.3 min is definetly low enough. Both 25% for 0.2 min and 35% with 0.3 min are used in experiments.

PDMS wie aussehen in Raumtemperatur

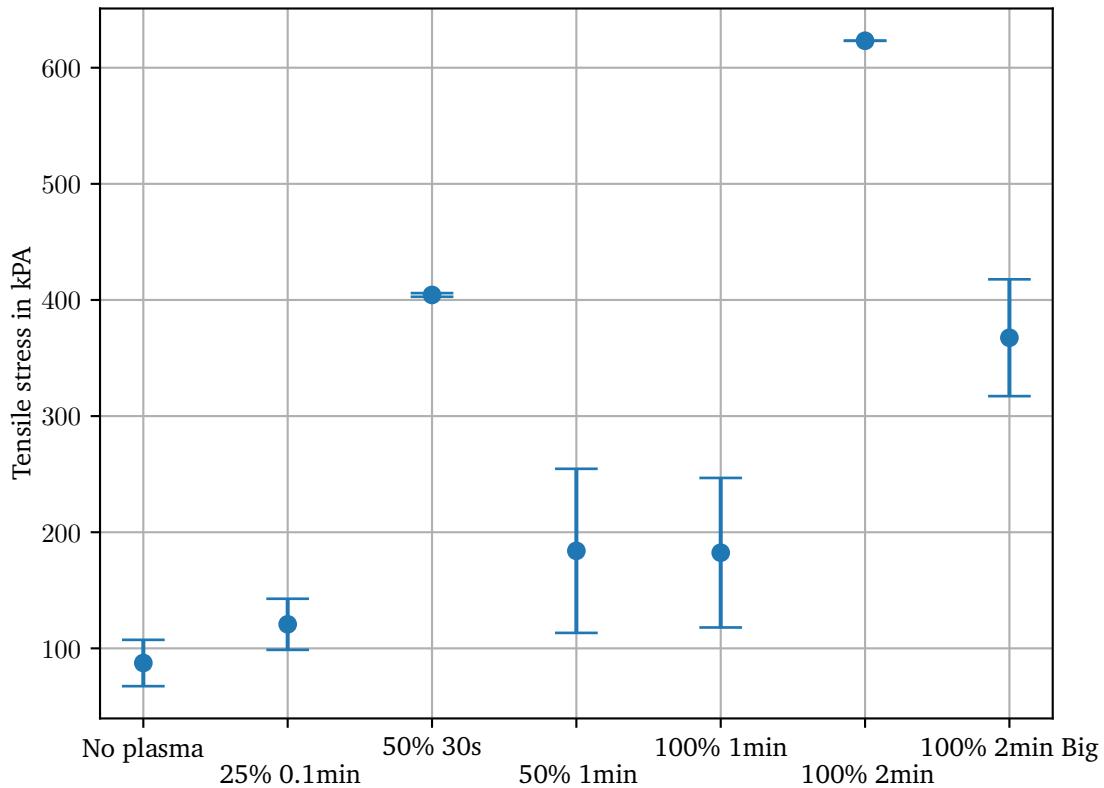


Figure 3.4: PDMS 2:1 Comparison between various Plasma curing strengths and durations.

3.3.2 experiments at cryogenic temperatures

At cryogenic temperatures, three different mixture ratios are tested. 1:2 mixture ratio is used with minimal plasmaactivation, based on the result in previous chapter. 4:1 is used with 10 minutes 100% plasma activation. 50:1 is used with 3 minutes plasmaactivation with 25% and 100%. additionally for 50:1, plasma activation for 10 minutes at 100% is tested

1:2

To additionally measure the force applied of the finger, the pulling machine is used in combination with the finger. In the lab, the small bath is used for sample preparation. after microscopy, the sample is transported to the pulling machine. the big bath is fixed on the bottom of the pulling machine. on top, the finger is clamped into the shackle and aligned to the shuttle. The process is still as previously stated.

This setup has a lot of limitation. Aligning the finger is very difficult, as the screws are hard to reach in cold temperature. When cooling, the previous alignment ist lost as the coldness distort the setup. Also little movement at the finger has maximum effect on the sample and alignment. touching the tubes can loosen the sample and/or the setup needs to be new aligned. Even a bigger window is not enough. Errors in alignment and therefore gluing to the border of the window and loosening through movement are the two biggest error sources in this setup. So the old setup is used in further experiments to eliminate big error sources.

Over all pulls, the HFE can transfer a maximum force of 1.30 ± 0.49 N. The area which experienced the force is unknown. Additionally, the force distribution is uneven (e.g. like Fig. 3.5a). Still, a worst case and a best case which are easily calculated can be determined. As best case, all force is evenly distributed under the area of the finger (Fig. 3.5b). With this assumption, the Area is equal to the finger tip area. The worst case is an even distribution over the whole surface (Fig. 3.5c). The surface is clamped by the window. So the inner diameter of the Window is the worst case area. In reality, the Force is somewhere in between (Table 3.4).

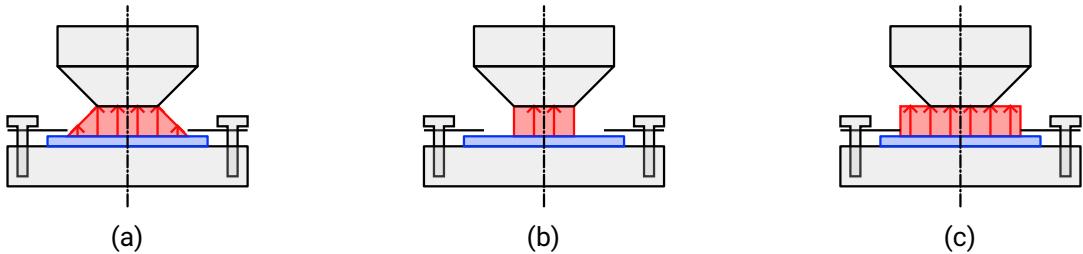


Figure 3.5: Areas Vgl (TODO BESCHREIBUNG)

Area used	Area Size	Tensile Stress
Finger	2.217 mm^2	$585.4 \pm 219.4 \text{ kPa}$
Small Window	4.91 mm^2	$264.3 \pm 99.1 \text{ kPa}$
Big Window	12.56 mm^2	$103.3 \pm 38.7 \text{ kPa}$

Table 3.4: different Areas used estimation of finger. The Real value is between the optimum of the finger area and the worst case of the window.

GRAPHIK HIER MIT MAXIMALER KRAFT DIE MIT HFE AUSGEWIRKT WIRD
RECHNUNG WIE DIE ZUGSPANNUNG IST ZWISCHEN DEN FENSTERN.

Zugmaschine mit Finger

4:1

50:1

4 Conclusion

so und tschüss

4.1 Lipide

4.2 Finger

4.3 PDMS

4.4 Ausblick

5 References

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