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Paper

FEASIBILITY OF SOFT TISSUE PREPARATION USING HIGH-PRESSURE WATER JET TECHNOLOGY

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ABSTRACT

The preparation of living tissue from human bodies is of major importance for investigations on bodily functions in preclinical research. Most studies use animal tissue or immortalized human tissue. The results of these studies can only be limitedly applied to the human body. It is therefore of great interest for research to make living human tissue available for research. For the preparation of such tissue, specimens from resections have to be used. A central problem is the availability of resected tissue parts for biomedical basic and therapeutic research. The preparation of soft tissue is a great challenge due to its fragility and its unstable consistency. According to the current state of art, the first step of soft tissue preparation is a tissue-stabilizing instillation of resected parts. After the instillation, the tissue can be cut into slices by a vibratome. For a wide range of investigations, the tissue stabilization limits the possibilities of investigations, since cavities are instilled. To investigate the bronchial system for example, it is instilled with low-melting agarose, which has to be dissolved after the transection, which limits the investigation to the parts with large lumina. To overcome such disadvantages of soft tissue preparation, we have investigated an innovative method of cutting porcine lung tissue transections by means of waterjet cutting. It enables the atraumatic cutting of slices with a high degree of plane parallelism and a thickness of less than 500 µm.

1 INTRODUCTION

According to the current state of art, preclinical research on bodily functions in human or animal organisms is executed on extracted cells outside its natural tissue context or immortalized tissue parts. The transferability of the results to the living human organism is often low [1]. Examination methods for research on living tissue are very limited. Living human tissue is very rare but also living animal tissue has a limited availability for research [2]. The reason for that lies in the preparation of those specimens. To observe bodily mechanisms, optical microscopy techniques are used very often. To gain a microscopic view on the specimens and keep the tissue alive, it has to be prepared and cultivated in a defined manner. Although the preparation is necessary to investigate the tissue specimens, it limits the examination options technically. Thin tissue slices have to be cut for the cultivation. The available preparation techniques use vibrating blades to cut the resected specimens into these slices. To allow a clean cut and to get a smooth cut surface, the tissue is instilled with stabilizing media before cutting [3, 4].

In the context of this study, the feasibility of soft tissue transections by means of high-pressure water jet technology was investigated using porcine lung tissue. Due to its cavities and its spongy texture, lung tissue, like other soft tissue types, places high demands on the water jet process. For the mechanical preparation of lung tissue, low-melting agarose is used to fill the alveoles and to stabilize the tissue [3, 4]. The agarose fills the alveoles and limits the feasible analytical methods. The removal of the agarose after the transection is complex [5] and possible only in large-lumen parts, which limits the research to these areas. The concept of using a high-pressure water jet for the tissue transection is that the water jet applies low loads to the tissue, compared to mechanical cutting tools. Because of this low load, we should be able to dispense with a tissue stabilizing instillation.

As the institute of materials science has a wide range of experience in the field of water jet applications in biomedical engineering, we investigated the feasibility of soft tissue preparation with high-pressure water jet technology at our water jet laboratory. For example, we have formerly designed a water jet process as an osteotomy tool [6] and developed and manufactured myocardial stabilizing structures [7] and structures for the aorta replacement [8] using an abrasive water jet process.

To make resected tissue available for research by avoiding a tissue-stabilizing instillation, in cooperation with the Department of Infectious Diseases and Pulmonary Medicine, we developed a new method for high-precision tissue transections using high-pressure waterjet technology.

2 PROCESS REQUIREMENTS

The preparation of the soft tissue must be designed in such a way that microscopic examination can then be performed as best as possible. For this it is necessary that the samples have a homogeneous surface and a high degree of plane-parallelism. When cutting, the unsteady texture of the tissue is a major challenge. It is therefore necessary to gather a basic understanding of the behavior of soft tissue that is manufactured by a water jet. We decided to representatively use porcine lung tissue for our investigations. Porcine lung tissue has a high availability and has a spongy texture with a lot of cavities. For preclinical research on the immune system, the preparation of lung tissue is highly significant. Porcine lung tissue is very similar to the human lung, which is why our results can be transferred easily on the preparation of human lung tissue. It was to determine if the lung tissue can be cut reliably. To realize high-precision tissue cuts, three main points are decisive for the success:

- Homogeneous cut surface: To investigate the tissue specimens with a high depth of field and to make sure that none of the thin slices is teared apart during the process, it is of major importance to develop a process that cuts the tissue to plane-parallel slices with a homogeneous surface.

- Fixation of the soft tissue: To ensure a reliable cut, it is necessary to provide a safe fixation for the soft tissue. We investigated different strategies for the fixation.

- Low loads on the soft tissue: So that the two previous points can be accomplished, it is mandatory to keep the loads on the tissue that are inevitably applied to it by the water jet as low as possible.

To keep the loads low and to be able to cut precisely at the site to be examined, we decided to use an orifice with a small diameter. The productivity of the process was negligible, as the following investigations will take place over several hours and therefore take a long time in relation to the cutting process. For this reason, we chose an orifice with a diameter of 0.04 mm. The use of a small orifice ensures a narrow kerf and because of the low flow, only low loads are applied to the tissue, compared to an orifice with a bigger diameter. To make sure, that we can realize a precise cut over the complete cut surface, we measured the expansion of the water jet as a function of the distance to the orifice to determine the maximum specimen dimensions we can cut with a nearly coherent jet. As shown in Figure 1, the jet is highly coherent in a distance up to 30 mm from the orifice. We therefore concluded, that we will be able to precisely cut specimens up to a height of 30 mm.

Next to the use of an orifice with small diameter we tried to keep the jet’s velocity low. The tissue is affected by venturi effect, resulting from the fast flowing fluid during the cut. The tissue tends to get sucked to the direction of flow into the kerf. This effect increases witch increasing flow velocity. The flow velocity depends on the water pressure, provided by the high-pressure pump. For that reason, we tried to keep the water pressure as low as possible while observing a satisfactory jet geometry.

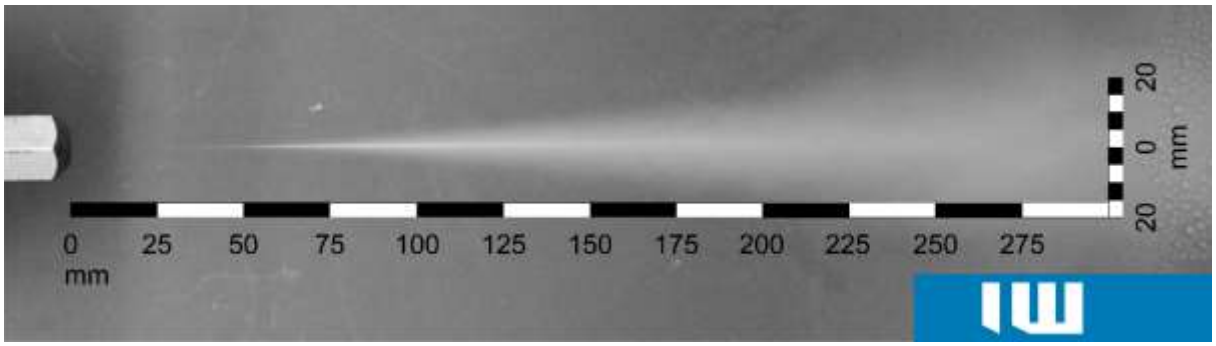

Figure 1. Water jet expansion at 70 MPa with a diamond orifice (d = 0.04 mm)

We repeated the measurements of the jet's geometry with pressures from 50 to 200 MPa by observing a maximum jet diameter above all pressures of 0.5 mm at a distance of 30 mm. This free jet's expansion is sufficiently low to ensure precise cuts. We identified the lowest expansion of the water jet around a pressure of 70 MPa, which is why we set a pressure range between 50 and 100 MPa for following test cuts.

3 TISSUE FIXATION

A safe fixation of the tissue is mandatory for the success of the cut itself. We tried to keep the fixation as easy as possible but the fixation of the specimen is a major challenge due to its spongy and soft texture. The lung tissue is very fragile because of its high amount of cavities surrounded by soft tissue. First experiments have shown that a mechanical fixation by piercing the lung provides a satisfactory result during the first cut. After dividing the lung in two pieces, no further fixation of the cut surface is possible, as shown in Figure 2. While cutting for the second time, the slice starts to wobble which has a negative effect on the plane-parallelism and the minimum thickness of the slice.

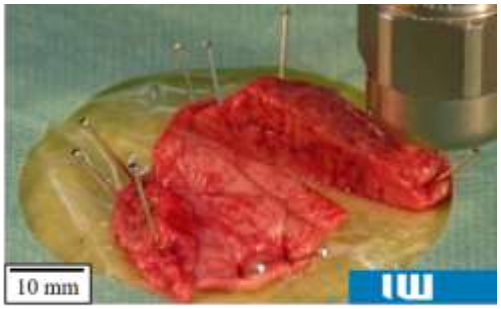

Figure 2. Test cut of porcine lung tissue using mechanical fixation.

To prevent the tissue from moving during the second cut we investigated two different fixation strategies. One uses low pressure over which the specimen is aspirated flat. The other approach uses a die cutter to pierce the lung and get cylindrical tissue specimens, which can be cut into slices afterwards. To cut slices from the cylindrical specimen, the tissue remains within the die cutter, which is fixated in a wedge-shaped clamping (see Figure 3).

Figure 3. Mechanical fixation device with die cutter.

To investigate the mechanical fixation strategy using the die cutter, cylindrical specimens are die-cut from outer parts of the porcine lung. We used the outer parts, because there are relatively low cavities, which is why the tissue from the outer parts of the lung is slightly more stable. We placed the die cutter with the die cut tissue in the clamping and tried to cut thin slices from it. We first cut a slice to get a plane cylindrical end face. The second cut was to create a thin cylindrical slice.

It showed up relatively fast that the force that is applied to the tissue by the water jet causes poor cutting results. In addition to the impulse of the jet, the specimen is affected by the venturi effect, generated through the fast flow of the water jet. The cut slice gets sucked into the kerf and can generally not be recovered for further investigations. The few slices we recovered did not know a satisfactory cutting result. As seen in Figure 4, the die cut cylindrical tissue specimens already show an unstable geometry due to the soft texture of the lung tissue. Thus, as well as the mechanical fixation by piercing the tissue, the mechanical fixation by die cutting cylindrical tissue specimens from the lung is not suitable for the preparation of high-precision tissue slices.

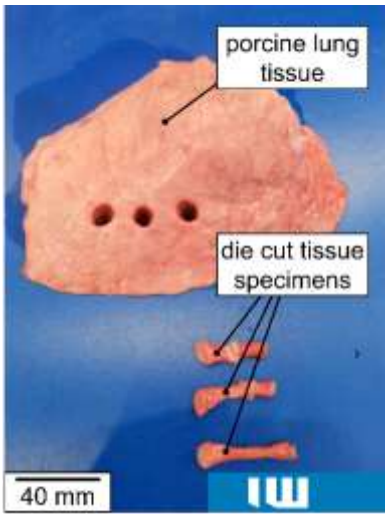

Figure 4. Die cut tissue specimens from porcine lung tissue.

Because a mechanical fixation does not lead to satisfactory cutting results, we came up with an alternative fixation strategy. We decided to build a fixation device that uses low-pressure to aspirate the tissue specimen from two sides (see Figure 5). The device is equipped with two suction grids. One is aligned horizontally and one vertically. It aspirates the specimen from the bottom and its side. The suction grid's hole pattern is quite coarse. We wanted to avoid the holes from plugging that there is no risk that the specimen unfastens during the cut.

Figure 5. Fixation of tissue specimens with low-pressure suction

For the generation of low-pressure, we used a VIPS 8 Type ejector of the company Landefeld GmbH. It uses the venturi effect, which is the reduction of fluid pressure when a nozzle increases the fluids velocity. The fluid flow is generated by pressurized air with pressures up to 0.7 MPa and an airflow up to 200 l/min.

For carrying out test cuts, we attached the specimen to the grids, through which the low-pressure is applied. Due to its non-plane-parallel structure, we aligned the specimen just roughly. The first cut serves to provide a plane-parallel surface on which the sample can be aligned more precisely. After the first cut, we removed the section and attached the plane cut surface to the vertical grid. That ensures the parallelism of the both cut surfaces. Due to the deforming of the tissue in order to the applied low-pressure, a deviation of plane-parallelism will occur.

We could affect the deviation by adjusting the low-pressure. It is to determine from which critical value a fixation of the sample is no longer reliable. During the first test cuts, we

encountered two major problems with the cut slices: The tissue separates from the fixation device at high cutting pressures and the fixation causes nubs on the tissue surface.

The first problem addresses the suction of the water jet at high cutting pressures. As mentioned before, we observed a separation between tissue and its holder due to the suction of the water jet. Even with using the maximum air pressure for the ejector, this problem occurred from time to time at cutting pressures above 100 MPa. In order to that, we limited the cutting pressure to 100 MPa by keeping the ejection pressure at its maximum.

Figure 6. Nubs on the tissue surface caused by the suction through the perforated sheet

The second problem that showed up during the test cuts can be seen in Figure 6. The suction of the tissue holder causes nubs on the tissue surface, which remain after removing the tissue from its holder. These nubs emerge from the suction through the perforated vertical aligned sheet. They occur at the bottom side as well but are not mandatory because we want to gain a microscopical view of the cutting surface, which is aligned vertically. Despite the fact, the surface quality does not match the requirements yet, we could state a fundamental feasibility of the tissue fixation using low-pressure suction.

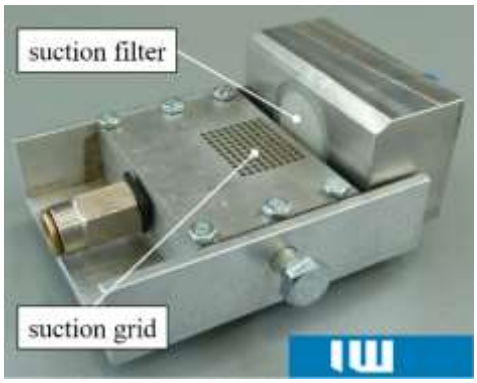

Figure 7. Modified fixation device with suction filter

To avoid the nubs on the tissue surface, we changed the perforated sheet for the vertical fixation with a commercially available suction filter of the company Fisher Scientific Inc. Figure 7 shows the modified version of the fixation device. The modification of the device by removing the perforated suction grid and installing a suction filter instead leads to a higher risk that the suction could plug. We therefore designed the holding that the suction filter is an easily replaceable part. To avoid clogging, it is replaced before every single cut.

The suction filter has a fine pored structure that leads to a uniform allocation of the suction force at the tissue surface. With the usage of the suction filter, we observed no more nubs. Next to avoid the suction filter to clog, a replacement of the suction filter against with a filter of another mesh can be useful to fix other tissue like liver or spleen.

4 CUTTING RESULTS

To evaluate the cutting result, we carried out further test cuts with porcine lung tissue. After cutting tissue slices, we used a confocal laser scanning microscope to get three-dimensional geometric data of the slices and to contactless measure thickness and plane-parallelism. Initially, we did not focus the minimum slice thickness but the homogeneity of the cut surfaces. After designing the process to achieve homogeneous cut surfaces, we minimized the slice thickness.

Resulting from the measurement of the jet's expansion, we varied the cutting pressure between 50 and 100 MPa. Compared to common water jet applications, this pressure range is quite low, which is advantageous for the application of soft tissue cutting. As described before, the low cutting pressure leads to a low flow velocity. Because of the low flow velocity, the suction from the water jet is low as well and we did not stand the risk of a separation of the tissue from the fixation device. As we presumed, a pressure of 70 MPa leaded to the most homogeneous cut surface. Although we kept the cutting pressure to a minimum, we mentioned that the water jet causes a vibration of the tissue, which leads to an undulated cut surface. To damp the vibration we additionally placed a plate made of cellulose nitrate under the tissue specimen, which successfully decreased the vibrations (see Figure 8). All further cuts were performed with a cellulose nitrate plate under the tissue specimen.

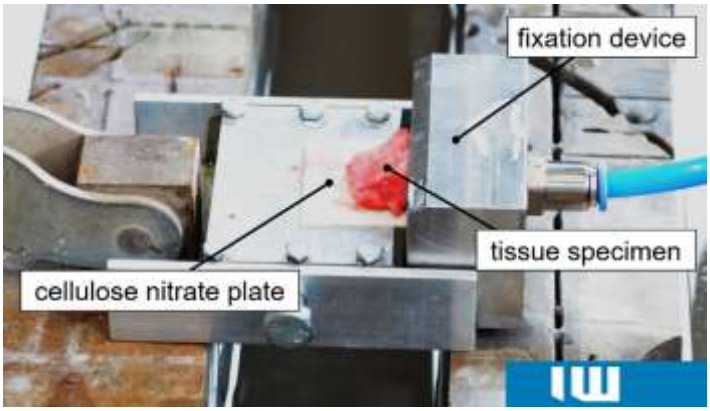

Figure 8. Porcine lung tissue attached to the fixation device with cellulose nitrate plate.

In the next step, we investigated the optimum feed speed while cutting with a pressure of 70 MPa. We varied the feed speed between 0.17 and 5 mm/s. To evaluate the cutting result, we determined the tissue's condition microscopically. The investigations showed up, that feed speeds below 2.5 mm/s lead to an atraumatic cutting surface. The higher the feed speed, the more damage could be observed at the cutting surface. Cutting extremely slow leads to disadvantageous surface qualities as well, as the tissue influences the water jet more often due to its small statistical movement. The optimum feed speed was between 0.42 and 1.17 mm/s. Figure 9 shows a cut slice and a picture taken with the laser-scanning microscope.

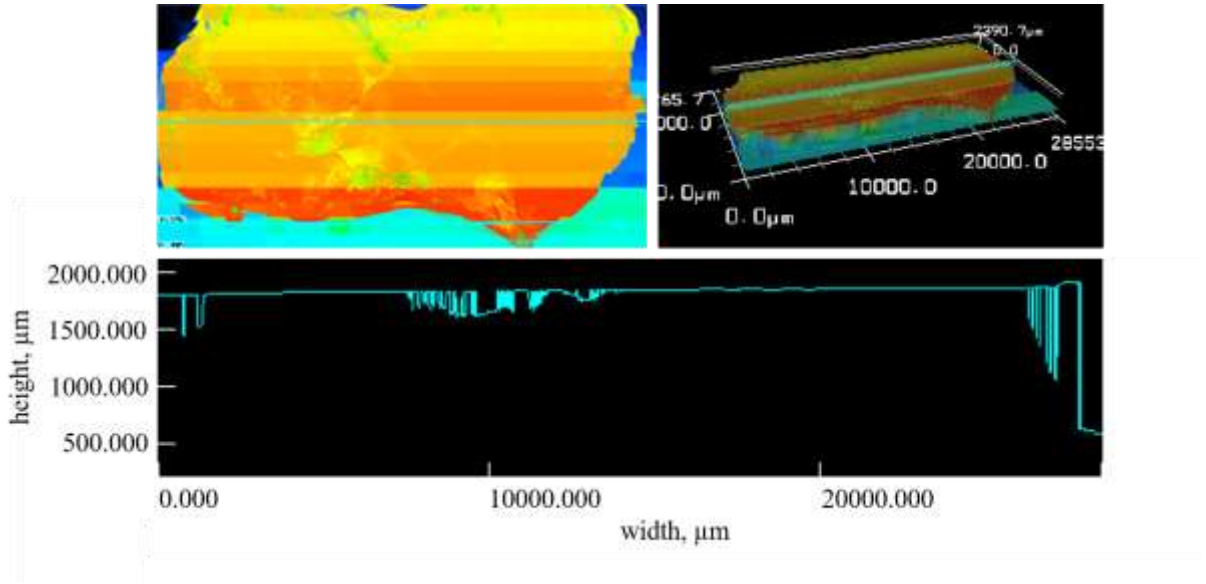

Figure 9. Three-dimensional view and height profile of a slice of porcine lung tissue taken by a laser-scanning microscope of the type Keyence VK-9710, v_f = 1 mm/s, p = 70 MPa

Figure 9 shows the profile of the cut slice. The upper pictures show a three dimensional view of the slice, the bottom picture shows a height profile over the complete width. It can be seen, that the slice is sufficiently plane-parallel with a cragged area on the cut surface. Despite the cragged area, there is enough plane surface for a microscopic view at the tissue. The plane-parallel surface allows further investigations with different optical microscopy techniques with gaining a high depth of field.

To keep the tissue in its vital state, it will be necessary to cultivate the tissue after the preparation. It is of great manner for the cultivation to cut thin slices. The tissue has to be perfused with special fluids to simulate its natural habit and to keep it alive. The goal was to cut slices thinner than 500 µm. The slice shown in Figure 9 has a thickness of about these 500 µm, which fits the requirements already. From this point, we cut increasingly thin slices by maintaining the process parameters and just repositioned the cutting path closer to the vertical low-pressure suction to determine the possible minimum of slice thickness with our process.

By performing further cuts, we managed to lower the slice thickness from 500 µm to below 200 µm. We cut even thinner slices, but not reliably. Within our experiments with porcine lung, failing to cut a slice from the tissue was not a major problem. Considering the poor availability of resected human tissue, the reliability of the cutting process is of paramount importance. Keeping this in mind, we conclude that we excelled our goal of cutting highly plane-parallel soft tissue slices with a thickness of less than 500 µm by managing to reliably cut slices with a thickness of 200 µm.

5 CONCLUSIONS

The goal of this research project was to prove the feasibility of atraumatic cuts of biologic soft tissue by means of high-pressure water jets. We exemplarily used porcine lung tissue for our investigations. Due to its spongy and soft texture, the preparation of lung tissue faces many problems. According to the previous state of science and technology, several compromises had to be made, to be able to cut soft tissue for research purposes. The tissue had to be cut without infusing it with stabilizing media like low-melting agarose. The slices should have a thickness below 500 µm and a high grade of plane-parallelism.

These goals could be fully achieved and even excelled. We built a device for the fixation of soft tissue with little damage using a low-pressure suction. This enabled us to atraumatically prepare porcine lung tissue for scientific investigations. The minimum slice thickness we managed to achieve was about 200 µm. The grade of plane-parallelism was sufficiently high.

As a next step, we want to transfer our results on cuts of other biological tissue like liver or spleen. Furthermore it will be necessary to develop a system that enables to cut within a sterile environment with a sterile medium.

The preparation of high-precision soft tissue transections leads to possibilities of cultivation of native living soft tissue. This creates the basis for observing human or animal bodily mechanisms using optical microscopy techniques in real time. Not relying on examining immortalized tissue or cells outside of its tissue context holds a great potential for major medical advances, such as in autoimmune research.

6 ACKNOWLEDGEMENTS

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