Microcutting of Living Tissue Slices and Stem Cell Colonies by Using Mechanical Tool and Liquid Jet

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***Abstract*—There is a growing need for methods to cut living tissues in vitro and cell cultures in microscale in biological and medical research. This paper presents two different microrobotic methods for cutting: mechanical microdissection using a sharp needle and liquid jet cutting utilizing a pressured liquid jet. Test devices for both the methods were built and the experiments were conducted with thin tissue slices and stem cell colonies. The devices built as well as the structure of the experiments and the results gained are discussed in this paper and the methods are compared with each other.**

I. Introduction

THE use of automatic microrobotic technologies is well established in the injection of living cells. Especially, for the manipulation of oocytes but also for the manipulation of living adherent cells, there has been lot of research activities within the recent years [1], [2], [3], [4], [5], [6], [7] and [8]. However, microrobotic manipulation of living tissue slices and stem cell colonies has not gained as large attention in the microrobotic society, despite the significant increase in the importance of tissue slice and stem cell colony applications.

In advanced in vitro cell models, one approach is to cultivate tissue samples obtained from a patient and conduct experiments on the primary tissue to study effects of compounds. To achieve this, tissue pieces are sliced and the areas of interest are microdissected. Suitable slicing machines are available, but automatic dissecting methods of thin living tissue slices are needed to be developed. New technologies are needed especially for applications where the dissected cells must stay alive in the dissection process.

New non-invasive cutting methods are needed also in the field of stem cell research. Cultivation of human embryonic stem cells (hESC) requires dissociation of cell colonies to prevent undesired differentiation. The process requires manual labor and is often done mechanically using surgical knives. In order to increase the automation level and to improve accuracy of the separation, it is essential to develop the dissociation methods.

Two techniques have become common in tissue microdissection in vitro: mechanical microdissection used e.g. in [9] and [10], and laser microdissection [11]. Also use of liquid jet cutting has been studied. The same techniques could be suitable for dissociation of stem cell colonies as well, but have not yet been reported.

In this work, living tissue slices and stem cell colonies are cut using mechanical microdissection and liquid jet cutting, and the results are compared. Even though the ultimate purpose is to cut an area out of a specimen and to move it out from the container, this paper concentrates only on the cutting phase and finding the most efficient cutting parameters for the both methods.

The methods were chosen because of the simplicity of the devices they require compared to lasers, the requirement for the dissection of living cells and the fact that the number of commercial products using these techniques is low despite the clear interest towards both of them.

Mechanical microdissection is performed using a micromanipulator and a sharp cutting tool. Manual micromanipulators and dissecting tools are widely available in the market, but MicroDissector PPMD (Eppendorf, UK) is the only commercial motorized device for mechanical microdissection of living tissue in vitro.

Liquid jet cutting incises the sample by ejecting liquid in a high pressure and it has been vastly used in industry. The technique has also been adapted to medical applications and its suitability has been studied in different fields [12], [13], [14], [15] and [16]. Yet, there is only one commercial device for liquid jet surgery (Helix Hydro-jet^{TM}, ERBE USA Incorporated) at the moment and it is not appropriate for cutting microscopic sections due to its relatively large nozzle size and diameter (120 µm). Good results and growing interest in the medical field have, however, given courage to experiment liquid jet cutting also in microcutting of living tissue slices, and the results have been promising stating the process precise and the amount of collateral damage small [17]. However, combining a liquid jet system with a microrobotic manipulator in order to automate the currently manual dissection of thin tissue slices and stem cell colonies has not been reported.

Section II describes the principles of the methods and the structure of the devices used in the experiments performed.

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Section III introduces the tissue and stem cell samples, and Section IV discusses the tests and the results achieved. Conclusions are drawn in Section V.

II. Methods

A. Basic Principles of the Methods

1) Mechanical Cutting: Mechanical cutting is performed by utilizing a micromanipulator and a mechanical cutting tool such as a needle or a knife. The cutting tool is attached to the arm of the micromanipulator and tissue or stem cell colonies are cut by pushing the tool inside the target and moving it horizontally. The tool can be vibrated to increase the efficiency of cutting. Also, some studies indicate that the damage done to the tissue is smaller when the tool is vibrating during cutting [9].

2) Liquid Jet Cutting: Liquid jet cutting slices material using a high-pressured liquid jet. The basis of cutting is micro erosion. Forcing a large volume of liquid through a small orifice causes the particles of liquid to rapidly accelerate. When the jet leaving the nozzle hits the target, the accelerated liquid particles touch a small area of the target producing small cracks to it. The eroded material is washed away by the jet and the cracks are further exposed to the liquid jet. The high pressure and the impact of particles result the small cracks to spread and deepen until the material is cut through. [18], [19]

The jet is generated when pressured liquid inside a chamber leaves the chamber through a narrow nozzle. The physical basis of the jet generation is the Bernoulli equation

$$p_1 + \rho g h + \frac{1}{2}\rho v_1^2 = p_2 + \frac{1}{2}\rho v_2^2 \quad (1)$$

where p_1 is the pressure inside the liquid chamber, h is the vertical distance between the tip of the nozzle and the chamber, ρ is the density of the liquid, v_1 is the velocity of the liquid inside the chamber, p_2 is the pressure of the liquid leaving the nozzle (equal to the pressure outside the tank) and v_2 is the velocity of the liquid leaving the nozzle. Since the velocity of the liquid inside the tank is negligible compared to the velocity of the jet and h is small, (1) can be simplified to

$$p_1 = p_2 + \frac{1}{2}\rho v_2^2 \quad (2)$$

and the velocity of the jet leaving the nozzle is

$$v_2 = \sqrt{\frac{2(p_1 - p_2)}{\rho}}\,. \quad (3)$$

A flow rate is gained by multiplying v_2 with the cross-sectional area of the nozzle orifice.

The factors affecting the cutting rate and the quality of liquid jet cutting can be divided to nozzle factors, fluid factors and object factors. The nozzle factors consist of the tip diameter and the distance between the nozzle and the object [18]. The former influences the flow rate and the latter influences the energy distribution of the jet and the velocity of the jet hitting the object [18], [20]. The fluid factors comprise fluid velocity, flow rate, pressure, viscosity and density [18]. The fluid velocity and the flow rate depend on the pressure and the tip diameter as discussed before. The two last factors have an effect on the success of jet generation and possible clogging of the nozzle. [18] The object factors contain the mechanical properties of the target material, the thickness of the target and the velocity, at which the target is moving under the jet [18]. The mechanical properties and the thickness affect how easily the target is cut. The moving velocity defines the time the jet has to drill through one point of a target.

B. Devices Used

1) Micromanipulator and Mechanical Tools: The micromanipulator used in the mechanical cutting tests was MANiPEN micromanipulator, which consists of two piezo benders and a linear stepper motor connected in series and operates on an inverting microscope [21]. The benders provide the horizontal movement and the motor the vertical movement. The arm of the micromanipulator forms a 45° angle with the horizontal plane. The manipulator is controlled using a tailor-made real-time software MART [22] normally used for cell injections and aspirations. The cutting tools used were Tungsten Dissecting Probes (50 mm long, 0.5 mm diameter rod, tip diameter smaller than 1 μm) manufactured by World Precision Instruments for tissue microdissection. The tools were attached to the arm of the micromanipulator using special adapters designed for them.

2) Liquid Jet Cutting Device: In the device, pressured air flows to a chamber filled with liquid and pushes the liquid out of the chamber through a microcapillary attached to it. The liquid chamber is fixed to a stand, which is fixed on an inverting microscope (Leica DM IL). A high performance CCD camera (Cohu series 4910) connected to the microscope is used for imaging. The target to be cut is placed inside a Petri-dish, which is attached to the manual x-y-table of the microscope. The distance between the capillary and the target can be adjusted using the stand, and the actual motion needed in cutting is made using the x-y-table. The liquid jet cutting device is shown in Fig.1.

The pressure source of the system is a cylinder of pressurized air with a pressure regulator (Aga Redline CR-series) connected. The pressure inside the cylinder is 200 bar and the outlet pressure can be adjusted from 2.5 to 50 bar with the regulator. From the regulator, air flows through a flexible steel pipe to the liquid chamber made of aluminum. The volume of the chamber is approximately 2.5 ml and it has a filling hole and a cap with a rubber seal to close the hole. In front of the chamber, there is a hole with M4 inner

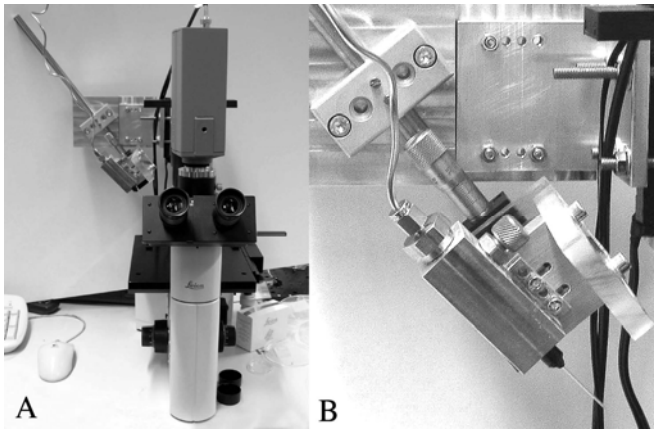

Fig. 1. The liquid jet cutting device. The device on the microscope (A) and a close-up of the liquid chamber (B).

threads for attaching a capillary. The chamber is fixed on a translation stage (Standa 7T173-10) and the translation stage is attached to the stand providing three degrees of freedom. Rough positioning is performed using the stand and fine positioning with the translation stage.

The capillaries used in the experiments were mostly self-pulled pipettes made using Sutter Instruments P-2000 micropipette puller and the inner diameters of their tips varied between 7 and 15 µm. The pipettes were pulled out of quartz glass having a 1 mm outer diameter and a 0.7 mm inner diameter (Sutter Instruments) and borosilicate glass having a 1.2 mm outer diameter and a 0.92 mm inner diameter (Clark Electromedical Instruments). Some experiments were also made with commercial micropipettes (Eppendorf Customtips) having an inner diameter of 20 µm. For attaching the capillaries to the system, special plastic adapters were used.

III. Samples

A. Tissue Samples

The tissue samples used in the experiments of mechanical cutting were human prostate slices having a thickness of 100 µm. The prostate tissue was obtained after informed consent from patients undergoing transurethral resection for benign prostatic hyperplasia at Tampere University Hospital and the specimens were sliced with the Krumdieck Tissue Slicer (Alabama Research and Development) in the University of Tampere Medical School [23]. The slices were cryopreserved in dimethyl sulfoxide -containing preservation medium at -80ºC prior to experimentation.

The tissue samples used in experiments performed with the liquid jet cutting device were 100 µm thick chicken liver tissue slices. Human prostate tissue could not be used, since it was not available when the liquid jet cutting experiments were made.

The chicken livers were acquired from a local poultry slaughterhouse and they were removed from the chickens ten minutes after death and stored in medium tubes, which were kept in ice. The tubes were then delivered to the University of Tampere Medical School and the liver slices were prepared as described in the previous paragraph.

During the cutting experiments, the tissue slices were kept in L-15 Leibovitz-medium (Sigma-Aldrich) in either Petri-dishes or well plates. To prevent the slices from floating and to keep them in place during cutting, they were glued on the bottom of the dishes using 2-octyl cyanoacrylate glue (Compeed liquid plaster) before adding the medium.

B. Stem Cell Samples

The hESC samples used were similar in both the cutting experiments and they were obtained from Regea, Institute for Regenerative Medicine (Tampere, Finland). In a single culture, stem cell colonies were growing on a fibroblast cell layer, which was attached to the bottom of a cell culture dish filled with a standard hESC medium described in more detail in [24]. The best and most promising parts of the stem cell colonies had been removed from the dishes with a scalpel for further cultivation before performing the cutting experiments. The stem cell samples were acquired from Regea on the same day the experiments were performed.

IV. Experiments and Results

A. Mechanical Cutting

1) Cutting of Tissue: Tips of the tungsten needles used in the cutting experiments narrow from 0.5 mm to less than 1 µm in 4 mm length. Therefore, the needles have very sharp tips but otherwise they are round. Thus, the effective cutting length of the needles is very small compared to the thicknesses of the tissue slices. To improve the cutting efficiency, the needle was vibrated by applying a triangular voltage signal to one of the piezo bender actuators. Then, the needle was pushed to the tissue and moved parallel to the direction of vibration.

The cutting experiments consisted of three steps: 1) the vibrating needle was pushed to a contact with tissue, 2) the needle was moved horizontally back and forth, 3) the needle was pushed further to the tissue. The second and third steps were repeated until the tissue was cut through. The effect of the following parameters on the cutting result was observed: the vibration amplitude and frequency, the depth of pushes between horizontal movements, the velocity of the horizontal movement and the number of horizontal back and forth movements in a certain depth. The last parameter was studied, since the needle is not always inside the tissue due to the vibration and therefore one successful horizontal movement does not necessarily ensure that a uniform cut is made. Cutting experiments were performed to the direction parallel to the 45º angle of the needle and the parameters of cutting were studied only for that direction.

Table I presents the most effective parameters obtained experimentally for mechanical cutting of tissue. 700 µm long and 100 µm deep cuts were made approximately in 5 minutes by using these parameters. The amplitude of vibration was remarkably high, almost 250 µm, and a

TABLE I
THE MOST EFFECTIVE PARAMETERS FOR MECHANICAL CUTTING OF TISSUE

Parameter	Value
Vibration amplitude	~250 µm
Vibration frequency	40 Hz
Horizontal velocity	15 – 20 µm/s
Depth of pushes	20 µm
Number of horizontal back and forth movements	1

triangular excitation signal with amplitude of 10% of the voltage range was used to produce it. Also, lower amplitudes of 5 % and 7.5 % of the voltage range were experimented but the vibration produced only oscillation of the tissue and no significant cutting result was achieved. The use of frequency of 30 Hz was almost as ineffective. The horizontal velocity used was maximum possible in the operation mode used in the manipulator. The depth of pushes was 20 µm, and one back and forth movement was sufficient to cut the tissue in a certain depth. Fig. 2 shows a cutting result in 100 µm thick prostate tissue.

2) Cutting of Stem Cell Colonies: The hESC colonies are very thin and therefore vibration is not needed in their cutting. Nevertheless, they are so thick and resilient that the needle tends to only push the cell mass ahead when moved if pushed too deep at first. This means that two pushes and two horizontal pulls are needed to cut the cell mass through. The direction of the horizontal pulls was the direction where the arm of the manipulator and the horizontal plane define a 45º angle, as with the tissue experiments. The horizontal velocity used was approximately 15 µm/s. Fig. 3 depicts cutting of stem cells using the needle.

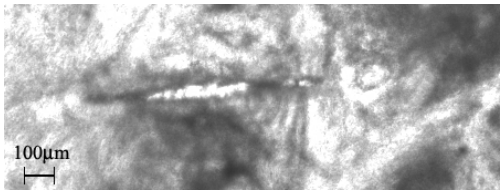

Fig. 2. A 750 µm cut made to 100 µm thick human prostate tissue using mechanical cutting.

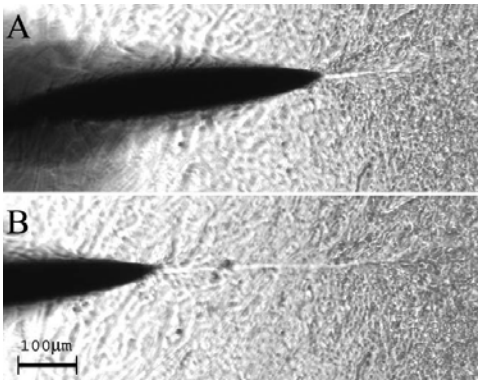

Fig. 3. Cutting the stem cell colony using mechanical cutting.

TABLE II
THE MOST EFFECTIVE PARAMETERS FOR LIQUID JET CUTTING OF TISSUE

Parameter	Value
Pressure	40 bar
Tip diameter	12 µm
Distance of the tip	< 1 mm
Horizontal velocity	120 µm/s

B. Liquid Jet Cutting

1) Cutting of Tissue: The liquid used in the cutting experiments was filtrated water because it keeps the liquid tank clean and does not stain it. This quickened performing of the experiments, since a separate cleaning step was not needed. As the goal of the experiments performed was to investigate if the liquid jet can cut the target, the possible damage to cells or tissues caused by water was not studied at this phase of research. Yet, the hypotonicity of water was not a problem. Water was filtrated by using membrane filters having a 0.2 µm pore size. The jet generation with filtrated Leibovitz-medium was also tested and proven successful.

The procedure used in the experiments was the following. First, the Petri-dish and the sample were fixed on the x-y-table of the microscope. Secondly, the distance of the capillary and the sample was adjusted. Finally, the pressure was applied to the system and the sample was moved horizontally under the liquid jet. The parameters the effects of which were observed included the pressure, the capillary tip diameter, the distance of the tip from the sample and the horizontal velocity. To observe possible partial clogging of the capillary, the duration of each jet was also measured and thus the possible changes in the tip diameter were detected.

Pressures from 20 bar to 40 bar and capillary tip diameters from 7 µm to 20 µm were studied. The distance to the sample was adjusted using the manual transition stage and measured with the naked eye. Therefore, the values are not very precise. Best results were gain when the distance was smaller than one mm but the capillary did not touch the surface of the sample. The velocity used was calculated from the lengths of the cuts and the time spent on cutting when cut as fast as possible. Table II presents the most effective parameters for liquid jet cutting of tissue. With lower pressures no cut was achieved and greater tip diameters only pressed, moved and sometimes even tore the tissue.

The cutting result is depicted in Fig. 4 and Fig. 5. As shown, the cut is wider and rougher than the cuts made using mechanical cutting. The width of the cut in Fig. 10 varies from 150 to 600 µm.

2) Cutting of Stem Cell Colonies: The liquid used in the cutting experiments of hESC colonies was also filtrated water for the same reasons as with tissue. The same experiment procedure was used as with tissue slices and the

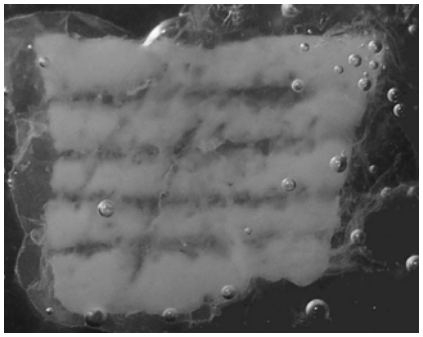

Fig. 4. Four parallel horizontal cuts in chicken liver tissue made by liquid jet. The length of each cut is approximately 9 mm.

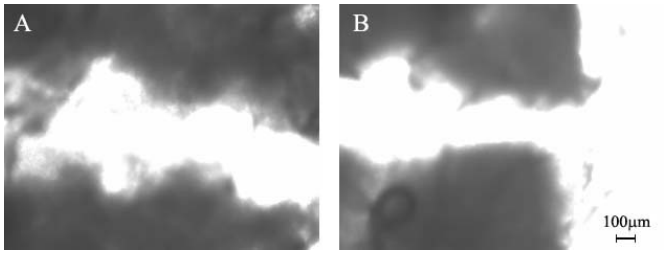

Fig. 5. Magnifications of one of the cuts in Fig. 4. The cut in the middle parts of the tissue sample (A) and in the edge (B).

effects of the same parameters were studied. The pressures experimented varied from 8 to 30 bar and the capillary tip diameters were from 7 to 20 µm. The velocities used were calculated from the videos taken and they ranged from 35 to 70 µm/s. Table III shows the most effective parameters for liquid jet cutting of hESC colonies.

With the highest pressures studied (20 bar, 30 bar), the jet blew the cell mass away instead of cutting. When the pressure was 10 bar, the cutting trace produced was wide and the jet still tended to blow the cell mass away. On the other hand, the pressure of 5 bar had not enough power for cutting. Increasing the tip diameter had the same effect as the pressure increase.

Fig. 6 shows the cutting result from two experiments. Similar to the tissue experiments, the cutting trace is wider and less accurate compared to mechanical cutting.

3) Common Problems: The ultimate problem encountered in the liquid jet cutting experiments was clogging of the capillary. In 75 % of the experiments made, the capillary clogged at least partly at some point of the experiment. Complete clogging is naturally detrimental and terminates the experiment but partial clogging is also harmful, since it reduces the flow rate and thus decreases the power of the jet.

TABLE III
THE MOST EFFECTIVE PARAMETERS FOR LIQUID JET CUTTING OF STEM CELL COLONIES

Parameter	Value
Pressure	8 bar
Tip diameter	10 µm
Distance of the tip	< 1 mm
Horizontal velocity	35 – 70 µm/s

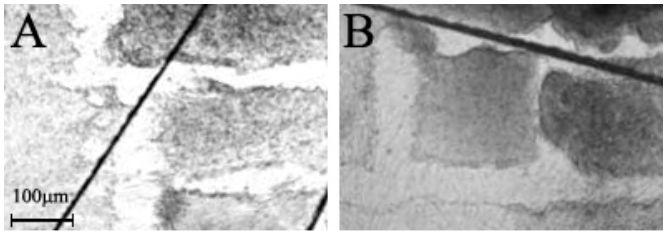

Fig. 6. Cuts made by liquid jet in stem cell colonies.

The most probable sources of impurities were room air, the liquid chamber and the plastic adapters of the capillaries. The chamber was cleaned regularly with pressurized air but some particles might still remain in the chamber. The adapters were cleaned with pressurized air before use but pushing the capillaries through them could have been removed particles from inner walls of the adapters. Design of new adapters and connection methods should overcome this problem.

V. CONCLUSION

This paper presented two microrobotic approaches for microcutting of living tissue slices and stem cell colonies: mechanical cutting and liquid jet cutting. Mechanical cutting was performed using a micromanipulator and a tungsten needle. In cutting experiments of tissue, the needle was vibrated parallel to the cutting direction to achieve a higher cutting power. In liquid jet cutting, a high-pressured water jet was utilized as a scalpel. The jet was produced by pushing liquid through a very narrow capillary using pressured air.

1*) Speed and width of cut:* The cutting result achieved using mechanical cutting was more precise but the cutting speed was significantly lower compared to liquid jet cutting. The cutting speed of 15 – 20 µm/s was used with prostate tissue and 15 µm/s with stem cell colonies in mechanical cutting while the corresponding speeds were 120 µm/s and 35 – 70 µm/s when liquid jet cutting was used. The width of the cuts produced to tissue with mechanical cutting was tens of micrometers while liquid jet cutting yielded 150 – 600 µm wide cuts. When the target was a stem cell colony, mechanical cutting produced smaller than 10 µm wide cuts while the liquid jet cuts varied from 10 to 90 µm in width.

2) Reliability: Mechanical cutting is much more reliable method, since the use of the same parameters always produces similar results. With liquid jet cutting, clogging of capillaries is a crucial problem changing the capillary diameter and the flow rate during the cutting process.

3) Cutting direction: Cuts in different directions can be made with the same parameters when using the liquid jet cutting device, which is necessary when aiming to cutting and removing a section from a sample. With mechanical cutting, cuts in only one direction were studied and produced. Also, the use of a high amplitude vibration in mechanical cutting of tissue essentially limits the form of dissected patches to rectangular. The direction of vibration must naturally be changed parallel to the direction of

motion. In the micromanipulator device used in the study, different vibration properties are needed when cutting along different axes of the micromanipulator. Therefore, integration of a turning sample dish holder is proposed to facilitate the use of only one cutting direction.

4) Stress caused: While using mechanical cutting, significant shaking in tissues and stretching in stem cell colonies were detected. Similar effects were not observed in liquid jet cutting tests. This implies that the collateral stress caused to the target is smaller when liquid jet cutting is used.

5) Future work: Clogging in liquid jet will be solved by the integration of a filtration system. Both the cutting methods presented need a method to remove the cut section out of the sample. The aim of this work was only to study and compare different methods for cutting of living tissue and stem cell colonies. Another future task is automation of the cutting process. A feedback system could be based on the visual system in both of the methods. In mechanical cutting, also a force measurement from the piezo actuators would provide valuable information on the dissection process.

6) Future trends: Mechanical cutting and liquid jet cutting were chosen as the tools for this study, since there was a need to cut *living* tissue and cells, and traditional laser techniques have not been suitable for this due to their heating effect. However, modern femtosecond lasers do not have this problem and they have been applied to nanocutting of living organisms [25]. Thus, they could develop to future methods for microcutting of living tissue and cells if also their price will become more affordable for cell applications.

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