CHARACTERIZATION OF LIQUID JET INJECTION INTO TISSUE BASED ON OPTICAL COHERENCE TOMOGRAPHY

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SUMMARY

We present the combination of high speed video monitoring, force measurement and Optical Coherence Tomography (OCT) as a new method for the characterization of liquid jets for needle-free tissue injection applications. It is well known that physical parameters, such as jet velocity and jet diameter determine the penetration characteristics of liquid micro-jets into a specific tissue [1]. The optimum injection parameters vary significantly for different types of tissues. Therefore, we have used high speed video monitoring and force sensor measurements to characterize the liquid jet. The penetration into the tissue is then analyzed by Optical Coherence Tomography (OCT) [2]. This enables the general description of the specific jet's physical properties as well as the penetration characteristics with respect to a specific tissue. In the experimental study, jets with diameters of 100 µm and jet lengths from 6 to 18 ms (~ 2 to 9 µl) have been injected into colon tissue from pigs.

KEYWORDS

Needle-free jet injection, micro fluidics, optical coherence tomography, penetration of tissue

INTRODUCTION

There is an increasing interest in the improvement of needle-free jet injection devices for healthcare applications [3]. This implies the need of improved methods to characterize the characteristics of liquid jets penetrating into tissue. State of the art methods are based on measuring the pressure difference across the dispenser nozzle to characterize a jet prior to its penetration. Nevertheless, there is a lack of information about the real properties of the jet when hitting the tissue [1]. The injection depth and injection volume are commonly determined by histological cuts of the target tissue or estimated by experiments with transparent model materials such as agarose gel [3].

EXPERIMENTAL SETUP

The aim of this scientific research was to enable needle-free trans-endoscopic jet injection, in abdominal tissue [4]. Hence, the target layer of medical interest for needle-free injection is the tela submucosa, as illustrated in fig. 1. To ensure a minimal change in the tissue's geometry due to the impact of the incoming jet during the penetration experiments a holder for the tissue was used that enables to apply a reproducible, quasi homogeneous tensile strain to the tissue. The holder was realized by a circular shaped support ring creating a well-defined constant spring force acting from backside onto the tissue.

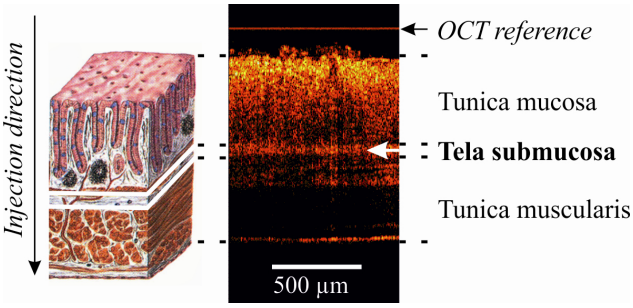

Figure 1: Physiology of colon tissue (schematic from [5]) in relation to an experimental OCT image. The tela submucosa - which is the target layer for injection - appears bright and is marked by a white arrow

For highly controllable release of liquid micro jets, we used a commercial valve (SMLD300G, F. Gyger, Switzerland) with a nozzle diameter of 100 µm. The valve was connected to a controllable pressurized reservoir, containing the injection fluid. The valve open times have been set between 6 and 18 ms, and driving pressures ranging from 5 to 60 bar have been used.

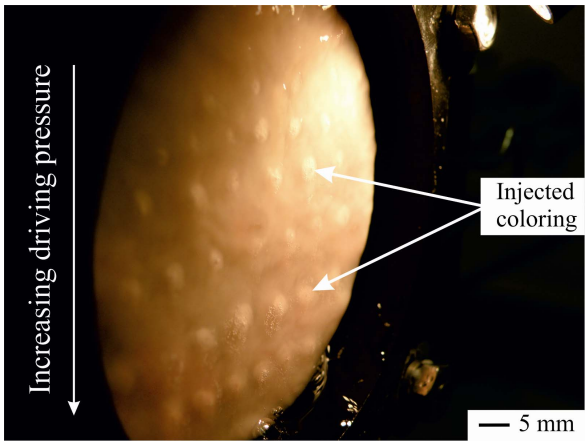

Figure 2: Colon tissue sample mounted on a circular holder after penetration experiments with red coloring solution. Every row shows injections at constant driving pressure. From row to row, the pressure is increased starting from 15 bar (top) to 50 bar (bottom). All injections are made at valve open times of 7.5 ms.

The valve was positioned in a distance of 5 mm from the freshly prepared clamped colon patches to inject a solution of red color ("Colour 7011", Trodat GmbH, Germany) at different experimental conditions. The coloring was used due to its optical properties to improve the contrast of the individual injection points on the tissue as well as the sensitivity of the OCT measurement technology used for subsequent analysis. A colon patch

subjected to different penetration experiments is shown in fig. 2 as example. It shows several needle-free injection points in rows of equal pressure realized by jets with different pressures in the range between 15 bar (top) to 50 bar (bottom).

For physical characterization of the ejected micro jets a force sensor (Type 9217A, Kistler Group, Switzerland, typical error ± 2% [6]) was used. As illustrated in fig. 3, the valve is precisely adjusted in 5 mm distance perpendicularly to the surface of the force sensor facing the center of the sensor. This enables to measure the force produced by the impinging jet over time as experienced by the tissue. For best correlation with the tissue experiments, the valve was left unchanged and the sensor was replaced by the colon patch, keeping the distance and all other experimental settings. Therefore, each injection condition (given by pressure and valve opening time) can be assigned to a certain force characteristics recorded by the sensor.

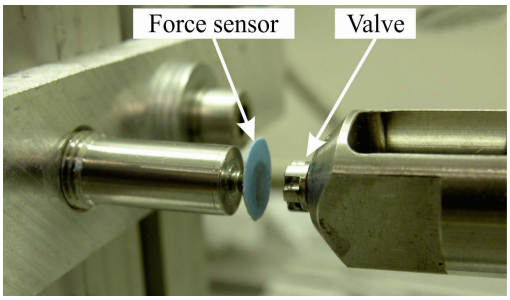

Figure 3: Experimental setup for force measurements

METHODS

In the following, the combination procedure of the three applied methods to characterize the liquid jets and their impact force regarding the ability to penetrate into the submucosa in a needle-free manner is given.

First, we characterized the jet by high-speed imaging and measured its time dependent impact force simultaneously. This enables to determine the physical parameters such as jet velocity and jet impact force as described above. Geometrical analyses of the micro-jets, recorded at a frame rate of 16,500 fps at a resolution of 288x210 pixels using a camera (MotionBLITZ, EoSens mini2, Mikrotron GmbH), helps to analyze the homogeneity of the jet's diameter over time and distance and characterize qualitatively the injection process.

Finally, OCT [2] provides a non-invasive depth-resolved two- or three dimensional measurement method of an injected sample in the tissue with a resolution of 20 µm in depth and 8 µm in transversal direction. Compared to investigations by histological cuts, this avoids errors by mechanical deformation of the tissue. These measurements were performed on colon samples fully immersed into NaCl-solution. The measurements were realized by a time domain OCT system [2], an interferometric method operating with low coherent light. Thereby an amplitude-mode-scan (A-scan, 1D) giving a reflectivity profile, provides depth information. By combining several lateral A-scans a 2D tomogram, a so-called brightness-mode-scan (B-scan), is created.

RESULTS

Fig. 4 depicts an example of the results gained by these experiments. It correlates individual images of an ejected jet at different times to the corresponding signal recorded by the force sensor. It is noticeable that the jet forms a little head at the beginning of the ejection process, as shown in fig. 4-B. This is caused by the inertial effect due the resting fluid that has to be accelerated to its final velocity. Therefore, the velocity of the jet is slower at the beginning and becomes faster over time carrying a little mass of fluid at its front. Due to the higher liquid mass at the head of the jet (fig. 4-B), an overshot of the impact force at the beginning (t = 0.5 ms) can be detected. The jet is fully established after t = 1.2 ms (fig. 4-E) and can be considered stationary on the sensor until t = 7.5 ms (fig. 4-F/G). Afterwards, the jet starts to decay due to the closing of the valve. After 7.5 ms, the tail of the jet breaks into single droplets (fig. 4-G) and ends after 7.8 ms (fig. 4-H).

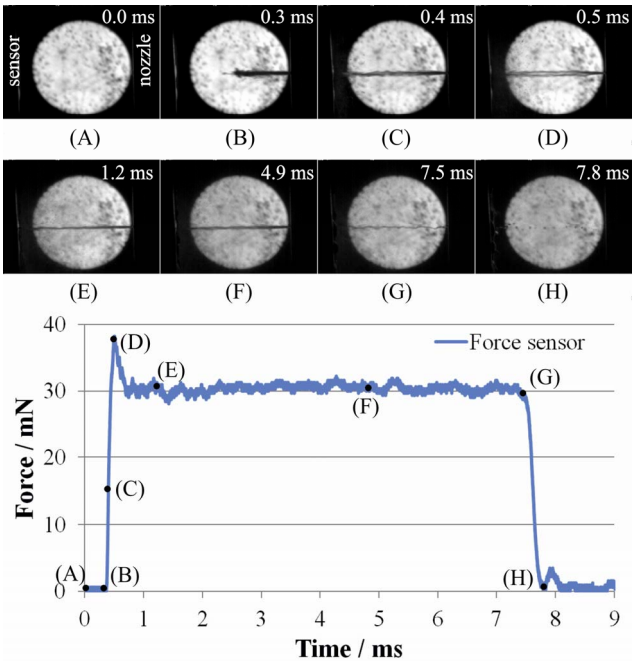

Figure 4: High speed pictures of a jet at 16768 fps in correlation to the measured force signal. Left side: force sensor, right side: nozzle; driving pressure: 38 bar

Considering the mean force value of the region between (E) and (F), where the jet diameter approximately equals the nozzle diameter d, a mean force of 24 mN can be extracted from the graph in fig. 4. The value of these average forces at stationary conditions over the complete pressure range considered in the experiments is shown in fig. 5. Based on these values the local pressure on the tissue can be calculated as follows: It is well known that jet diameter d, jet velocity v and jet impact force F are linked via Bernoulli's equation [7]

$$\frac{1}{2}\rho\, v^2 = \sigma_{crit} = \frac{F}{A} \quad with \quad A = \frac{\pi\, d^2}{4} \qquad (1)$$

where ρ is the fluid density, σ_{crit} the critical tensile stress of the tissue and A the cross sectional area of the jet.

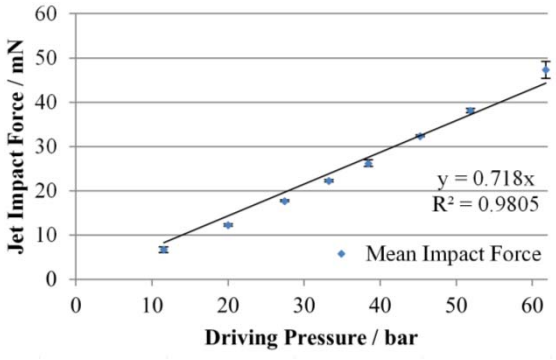

Figure 5: Measured average force produced by a micro jet which is driven by different pressures. The used valve contained a 100 µm nozzle and was placed in a distance of 5 mm. Jet duration of 7.5 ms was used. Each point represents the mean impact force out of 10 measurements. The error bars are given by the standard deviation.

Using equation (1) one can calculate the actually exerted pressure of the impinging jet referred to as *impact pressure* from the driving pressure. At a driving pressure of 38 bar for example a jet is ejected whose mean impact force on the substrate was measured to be 26.2 mN. According to (1), assuming a jet diameter of 100 µm one can calculate the corresponding jet pressure to be 33.4 bar. The difference between driving pressure and jet pressure can be explained by the resistive pressure drop over the valve and the fabrication tolerances of the nozzle (± 5 µm), as well as the measurement accuracy of the sensor (typical error ± 2% [6]). For the total experimental study the jet velocities are spanning a range from 55.8 m/s at 12.2 mN up to 89.5 m/s at 38.1 mN.

The described method was used to characterize the relevant physical properties of the jets before injection experiments, i.e. the time-dependent impact pressure, impact velocity and the jet length. This enables tissue penetration experiments with well-defined jet properties. In the experiments shown in the following, the focus was on the penetration characteristics of 100 µm jets into pig colon tissue. To conduct these experiments, the force sensor was replaced by the clamping fixture holding the pig colon samples. Highly reproducible jets with known impact forces (standard deviation of impact force < 2 mN) according to fig. 5 have been used for injection experiments at different driving pressure levels.

The results of injection experiments with fresh pig colon tissue (less than 2.5 h after recovery of the sample) are shown in fig. 6. For this experiment, a jet duration of t = 7.5 ms was used. Comparing the OCT measurement result of an unaffected piece of colon to its histologic scheme (fig. 1), the different layers as well as enclosed liquid volumes can be classified, as shown in fig. 6. The depth of the injection can be extracted from the images with respect to a reference line (cf. red line in fig. 1 and fig. 6). The white dashed lines mark the injected liquid quantity whereas the white arrows are indicating the tela submucosa. This layer should be penetrated to yield a successful injection for the considered medical application.

Starting at impact forces of 12.2 mN (fig. 6a) the smallest injected volume has been observed. In accordance to fig. 2, an increased force (pressure) at constant valve opening time leads to more injected fluid. However, in fig. 6c the 2D OCT measurement scan has not perfectly met the center of the injection. Therefore, the injected volume appears to be less than in fig. 6b. A similar situation can be observed in fig. 6e where the injection whole can be seen additionally but the main part of the injected fluid moved away in lateral direction into the tissue. Obviously, the shape of injected liquid provided by a line scan does not provide full information on the injected volume. Therefore, it would be preferable to use more OCT B-scans to visualize a 3D volume in future. Nevertheless, it is possible by the B-scans to determine the injection depth: Fig. 6f is showing the only successful injection into submucosa layer at a jet impact force of 38.1 mN, leading to the conclusion that jet impact forces higher than 32.3 mN (fig. 6e) are required for successful injection into this layer.

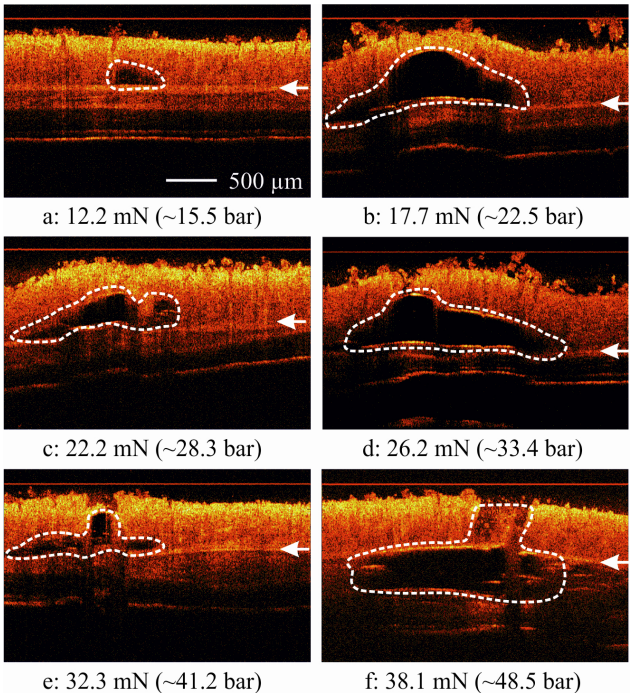

Figure 6: OCT images of injections produced at various impact forces (impact pressure in brackets). Dashed lines identify injected liquid volume. The submucosa is marked by a white arrow.

Due to the significant effort of harvesting colon tissue and using it for experiments without delay, all subsequent experiments have been made with frozen tissue of the same pig colon that was thawed again right before the experiment (storage time was less than two weeks at -8°C). When repeating the experiments shown before with thawed tissue, it turned out that under identical conditions the required minimum jet impact force for a successful injection into the submucosa layer is around 26.2 mN (~33.4 bar impact pressure) at valve open time of 6 ms (cf. fig. 7). Though, this is significantly less than for fresh tissue, the effect was expected, as due to the 'slow' freezing, growing ice crystals might destroy part of the

cell membranes [8] which can lead to reduced mechanical stability. Therefore, quantitative data for impact pressures and penetration depth obtained with thawed tissues cannot be directly compared to fresh or living tissue. Nevertheless, qualitative characterization of the injection process is certainly possible.

In order to study the influence of the jet length on the penetration depth experiments with different valve opening times of 6, 12 and 18 ms have been conducted. The corresponding measured volumes of the ejected jets were in the range of 1.3 to 9.1 µl. According to a theory from Baxter et al. [7], a critical tensile stress value has to be overcome to penetrate into the tissue. In this model, it is considered to be sufficient that the impact pressure at the injection point overcomes the local tensile stress of the tissue to penetrate into it. Thus, the jet duration should not be relevant for penetration success.

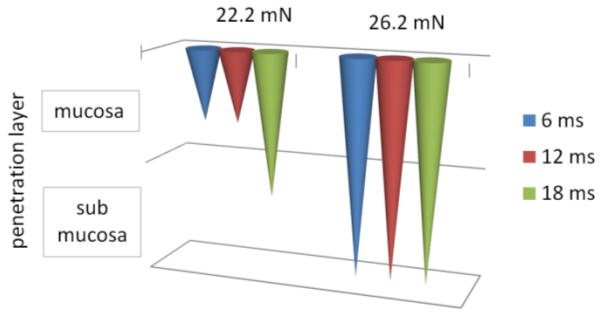

Figure 7: Experiments with frozen and thawed colon tissue (n=3). Different valve open times lead to the same results: At 22.2 mN, injection predominantly into the mucosa; at 26.2 mN, injection exclusively into the submucosa. For 18 ms at 22.2 mN, injection into either one of both layers was observed within 3 tested injections.

In fig. 7, two groups of jets with different impact pressure and jet length are displayed and the penetration depth is shown for each jet. The data was extracted out of three experiments at each setting using OCT images similar to fig. 6 to determine the penetration depth. Obviously, jets with 22.2 mN (~28.3 bar) impact force are not able to penetrate safely into the submucosa, while jets with 26.2 mN (~33.4 bar) penetrate into the submucosa independent of the jet length. Most interesting is the case of a jet at 22.2 mN with a jet length of 18 ms. At this experimental condition one injection was observed to reach only into the mucosa, one into the submucosa and one result was undecidable. Though, it is confirmed by these experiments that the impact pressure is the most significant parameter to control penetration depth, it still cannot be excluded that for certain settings close to the threshold of penetration, the jet length might play a role as well.

CONCLUSION

The measurement of micro-jet impact force in combination with high speed imaging and OCT analysis of the tissue enables a detailed investigation of needle-free injection experiments. On the one hand, the impact force measurements can be used to quantitatively characterize the physical properties of the jet in terms of jet duration and impact pressure. The high speed images complete this characterization by providing qualitative data on jet integrity and swelling of the tissue under impact. Both methods together provide a full characterization of the jet itself regardless of the device generating the jet. In particular, the jet impact pressure can be determined precisely to provide controlled experimental conditions for tissue experiments. The OCT technology on the other hand can be used to investigate the tissue subsequent to injection experiments. Using lateral line scans (B-scan), the penetration depth can be determined with reasonable accuracy (20 µm in depth; 8 µm in transversal direction).

In further research a faster OCT system using frequency domain transformation could enable the measurement of 3D volumes (C-scan) to determine the shape and amount of the injected liquid volume as well. With such an approach the injection process into various tissues could be characterized very precisely in the future.

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