Published in final edited form as:

Health Innov Point Care Conf. 2014 October ; 2014: 271–275. doi:10.1109/HIC.2014.7038927.

Feasibility of a hybrid elastographic-microfluidic device to rapidly process and assess pancreatic cancer biopsies for pathologists

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Abstract

In this study, our collaborative research group explored the possibility of incorporating ultrasound elastography technology with a microfluidic device that is designed to prepare fine needle core biopsies (CBs; $L=0.5\text{--}2.0$ cm, $D=0.4\text{--}1.2$ mm) for pancreatic cancer diagnosis. For the first time, elastographic techniques were employed to measure shear wave velocity in fresh (3.7 m/s) and formalin-fixed (14.7 m/s) pancreatic CBs. Shear wave velocity did not vary whether fixed specimens were free on a microscope slide, or constrained within glass microfluidic channels: 11.5 ± 1.9 v. 11.8 ± 2.1 m/s. 4% agarose inclusions were also embedded within 1% agarose hydrogels to simulate cysts, neoplastic, or necrotic tissue within CBs. Inclusions were successfully visualized and measured using optical coherence elastography. These preliminary experiments demonstrate in a rudimentary fashion that elastographic measurements of pancreatic CBs may be incorporated with our microfluidic device. The rapid mapping of CB stiffness may provide qualitative spatial information for pathologists to determine a more accurate diagnosis for patients.

*R. Das and T-M. Nguyen are co-first authors.

Seibel discloses co-ownership of US patent applications.

I. Introduction

High-throughput, low-cost devices which assist pathologists in rapidly determining an accurate diagnosis represent an R&D niche with wide-reaching implications for cancer pathology. Recently our research group demonstrated for the first time that a custom-designed microfluidic device can perform many of the rudimentary operations on core biopsies (CBs) similar to larger-scale, multi-person medical pathology laboratories^{1–2}.

A. Traditional pathology and core biopsies

To aid pathologists in cancer diagnosis, pathology laboratories obtain biopsies from patients in the clinic and then prepare and process them by 1) fixing samples, 2) staining appropriate biomarkers, or cellular targets, and 3) optically clearing processed specimens. Thin samples are then imaged and visualized in 2D on a microscope slide using simple brightfield optical microscopy.

Slides typically consist of a slurry of isolated cells (i.e., fine needle aspirates, FNAs), or tissue slices sectioned from larger specimens (CBs). When comparing multiple 2D sections of CBs, more 3D structural information about a disease in its *in vivo* state is retained. A fundamental gain in information is obtained altogether when comparing unsectioned, whole CBs to tissue slices (i.e., *in vivo* tissue architecture, 3D distribution of neoplasms, invasiveness and stromal response). This information is paramount for the diagnosis and study of pancreatic cancer which induces structural changes in tissue surrounding neoplasms³. In most patients, pancreatic cancer is diagnosed when the disease has already progressed to its final, most lethal stage. Early detection remains relatively nonexistent because the disease's development and pathology (by many expert accounts) are still relatively unknown^{3–5}.

B. Microfluidics and core biopsies

Current developments and proof-of-concept investigations concerning 3D imaging modalities^{6–12}, optical clearing agents (OCAs)^{13–18} and image processing algorithms^{19–20} have provided simple approaches to image and visualize entire, intact (i.e., unsectioned) CBs. Towards this aim, our device additionally fulfills the need to prepare and process small enough tissue specimens (CBs) for 3D imaging by utilizing microfluidic technology and fluid flow principles in a novel, but simple microchannel design and configuration^{1–2}.

C. Core biopsies and elastography

CB specimens are neither strictly microscopic, nor macroscopic. Typical clinically-procured fine needle CBs are 0.4–1.2 mm in diameter and 0.5–2.0 cm in length (~1–20 mm³). In comparison to the pancreas, these microscopic biopsies constitute ~0.03% of the total organ volume^{22–23}. The advantage of small diameter CBs is that the entire specimen can be chemically fixed in less than 20–30 min, if not faster²¹. However, even without OCAs CBs can also be rapidly inspected optically and macroscopically for suspect regions, which may then be subsequently imaged at higher resolution in either 3D, or 2D (after sectioning).

Entire intact CBs potentially may reflect the *in vivo* mechanical state (i.e., stiffness) of the pancreas and yield vital information for the pathologist. For example, manual palpation, which is based on tissue stiffness²⁴, is still widely used for screening of breast and liver lesions. A similar, but more objective assessment may be accomplished through non-invasive ultrasound elastography^{25–26}. In cancer, most tumors are stiffer and more dense than normal tissue^{27–28}; preliminary mechanical investigations have revealed that neoplasms and the surrounding stroma are mechanically distinct from normal, healthy cells^{29–30}.

D. A hybrid device?

Taking these issues into consideration, our collaborative team posed the following bioengineering question: *can elastography be combined with our microfluidic device in a hybrid fashion to maximize the utility of a procured CB?* That is after CBs are prepared and processed for pathology, is it feasible that elastography may be used to measure and map 3D stiffness data? Do these data correlate with the 3D optical distributions of cellular features?

Clinically, elastography is predominantly used on whole organs, or large (centimeter size) samples, thus the question if the modality can be applied to much smaller tissue (CBs), especially those enclosed within a microfluidic channel, is both novel and highly relevant. Non-invasive, non-destructive testing of CBs within our microfluidic device would neither reduce CB quality, nor add significant time within the flow of events towards 3D imaging. Therefore the hybrid method may be inserted within the pathology workflow without minimal disruption, or impedance.

E. Investigation

In this study, shear wave elastography based on optical coherence tomography detection (OCT-SWE) was used for the first time on pancreatic CB tissue. The geometry and dimensions of corresponding CBs matched typical samples procured by trained clinicians, or samples regularly processed by our microfluidic device. The investigation's objectives were the following:

1. can OCT-SWE measure the stiffness of small pancreatic tissue specimens (CBs) and
2. is it feasible for OCT-SWE to resolve distinct structures (i.e., tumors) within the inner volume of pancreas CBs.

II. Methods

A. Shear wave elastography

We used a phase-sensitive OCT-SWE setup to track shear waves induced in samples (fig. 1). Details about this technique may be found in previous publications^{31–32}. Briefly, a piezoelectric actuator was attached to a polished tip and used to generate a 1–7 kHz shear wave in CBs, or agarose specimens. The shear wave propagation was tracked at a frame rate of 44.8 kHz using phase-sensitive OCT. The local propagation speed of the shear wave was calculated at each pixel of the imaging plane using a custom-written time-of-flight algorithm. The shear wave speed (v) is known to be related to soft tissue stiffness (i.e., $E \sim 3\mu = 3\rho v^2$, where E = Young's modulus, μ = shear modulus and ρ = density)

B. Pancreas core biopsies / microfluidic channels

Pancreas CBs were procured using coring needles on fresh and formalin-fixed porcine pancreas tissue. Samples were then deposited within 1–2 cm long microfluidic channels (square glass capillaries, fig. 1a). Microchannels consisted of a rectangular (2×4 mm), or square (2×2 , 1×1 , 0.5×0.5 mm) cross section depending on the experimental condition. Square capillaries were chosen over cylindrical ones for their flat walls which facilitate OCT imaging.

Fresh and fixed CBs were also placed in a PDMS-based square (3×3 mm) cross section microfluidic channel (fig. 1d). The top surface of the microchannel consisted of a thin (< 0.2 mm), flexible PDMS membrane in order to ascertain if OCT imaging, or propagating shear wave was significantly impacted by the substrate.

To compare results, density was measured by taking porcine pancreas samples and cutting them into 1 cm^3 blocks. Tissue block volumes and masses were precisely measured using digital calipers and a precision scale. Shear modulus (μ) was back-calculated based on the measured density and shear wave velocity (i.e., $\mu = \rho v^2$).

C. Agarose phantom specimens / CB tumor detection

Healthy and cancerous (i.e., tumor presenting) human pancreatic CBs would have been ideal for the current study. However, time restrictions, stringent protocols for human subjects and lack of a pig cancer model forced our group to turn to agarose hydrogel phantom substitutes to simulate healthy and diseased pancreatic tissue. As a first step, 1–4% agarose hydrogels were made up and sectioned in 1 cm^3 blocks. Density measurements similar to the method with pancreatic tissue were recorded and tabulated.

Next, 1×1 mm square capillaries were filled with a 1% agarose hydrogel (based on previously found density data) to simulate fixed CBs. A 26 G coring needle (OD = 0.45 mm) was pushed through the interior of the hydrogel to core out an empty space; the same needle was then employed to fill the empty space with a boiling 4% agarose suspension, which instantly polymerized, thereby simulating a tumor, or diseased (necrotic) tissue (fig. 2).

The 4% agarose hydrogels also contained a 1% w/v concentration of 300 nm diameter latex microspheres to provide scattering for the OCT imaging modality. A similar method was used for the 1% agarose hydrogels in a secondary experiment; in this setup, a small 4% agarose inclusion was embedded within the 1% hydrogel. OCT imaging and elastographic measurements were then made of the (simulated) tumor embedded within the CB.

III. Results and Discussion

For the first time, both fresh and fixed pancreatic CBs were imaged and measured with OCT-SWE (fig. 3). Preliminary results demonstrated that shear wave velocity measurements of CBs were independent of microfluidic channel enclosure (fig. 1b).

Shear waves propagated incompletely and much slower through fresh pancreatic CB tissue (fig. 3-bottom). Intuitively, this made sense. While both fresh and fixed pancreas tissue are viscoelastic, fresh tissue is much more viscous and therefore poorly transmits shear wave energy across the CB length.

Shear wave velocity was at least 4x faster in fixed pancreas CB specimens than in fresh ones (fig. 4). In a case study³³, shear wave velocities in fresh human pancreas tissue were measured to be 1.43–1.51 m/s (2.5x slower). The discrepancy may be due to limited human sampling, *in vivo* measurements and/or anatomical differences between human and pig pancreas. In our study, we obtained a good repeatability (< 5% error) for our measurements.

Similar measurements were recorded for fixed specimens within the PDMS-based (fig. 1d) microfluidic channel. Qualitatively, the channel did not significantly attenuate light for OCT measurements and shear wave velocity data were similar when CBs were free, or enclosed.

Fixed and fresh pancreas tissue, and 1–4% agarose hydrogel densities were measured and tabulated (table 1). A linear regression performed on the agarose data permitted the determination of a suitable (%) agarose hydrogel substitute. Preliminary shear moduli results were produced when shear wave velocity data (fig. 4) were combined with measured CB density data (table 1). A shear modulus of 18 and 227 kPa was found for fresh and fixed pancreas CBs, respectively. To our knowledge, little to no scientific literature is available on the comparison of shear moduli between fresh and fixed pancreas tissue. In one analytical and experimental study³⁴, the shear modulus of fresh porcine pancreas tissue was estimated to be on the order of 100 kPa. However, results were unique since shear waves were generated at very low frequencies and in a geometry distinct from our current setup.

Finally, OCT-SWE was able to resolve and measure high concentrated agarose inclusions within a less concentrated microfluidic channel-enclosed agarose hydrogel (fig. 5). Fig. 5a is a macroscopic photograph (side view) of the 4% agarose inclusion along the entire length of the microfluidic channel. During the initial setup, the experimenter's hand was jostled such that the coring needle creating the empty channel for the 4% agarose had to be removed and then reinserted. Consequently, a small section of the empty channel had a doublet, which was successfully captured by the imaging system. The doublet found at the left most red vertical line in fig. 5a was resolved by the imaging system in fig. 5b (i.e., the cross section of the microfluidic channel at that line).

In fig 5d–e, a circular 4% agarose inclusion was embedded within a 1% agarose hydrogel to simulate neoplastic, or hardened necrotic tissue in a related, but crucial experiment. OCT-SWE was then used to measure the shear wave speed in order to differentiate the 4% mass within the microfluidic channel (fig. 5e). Qualitatively, measurement and identification of the stiffer embedded tissue within the CB in our microfluidic device is feasible and a distinct reality.

Acknowledgments

Research was supported by NSF CBET-1212540, NIH R21 CA186791, GAANN Image-based Biomechanics Modeling Program in the UW Mechanical Engineering Dept, NIH R01 EB016034, NIH R01 CA170734, NIH R01

EB009682, NIH R01 HL093140, NIH R01 DC010201, Life Sciences Discovery Fund 3292512 and the Coulter Translational Research Partnership Program in the UW Bioengineering Department.

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Figure 1.

OCT-SWE configuration with CBs enclosed in microfluidic channels. a) Several samples of fresh and fixed pancreatic CBs were prepared and positioned within glass microfluidic channels (capillaries) and held in place by putty in a petridish prior to experimentation. b) Yellow arrow points to the piezocrystal actuator, which made contact with the tip of a CB contained within a channel. Imaging was done from above. c) Yellow arrow points to the same actuator tip (side view) that was imaged from above by the OCT-SWE system. Left (blue) screen on the imaging system displayed the acquired image; right (red/white) screen displayed an image (phase sensitive) only when the actuator was actively generating a shear wave. d) CB within a PDMS-based microfluidic channel. Missing material at the arrow tip of “PDMS substrate” is a fabrication error. Large yellow arrow points to actuator tip, which

made contact with the CB at the open end of the channel (distal end at this angle). The proximal opening (i.e., small window cut out of the membrane) allowed the channel to be filled with fluid and prevented end-reflection of the shear wave.

Figure 2.

Simulated CBs containing simulated cyst, neoplastic, or necrotic tissue. Representative samples of square capillaries containing a 1% agarose hydrogel. Embedded is the denser 4% agarose (large yellow arrow). Samples were ~1 cm in length and 1 mm in diameter. Small yellow arrow denotes a trapped air bubble.

Figure 3. Shear wave velocity distribution in fixed (top) and fresh (bottom) pancreas CB samples. Fixed samples were more elastic in comparison to fresh ones since a distinct shear wave map was able to be visualized across the specimen. The source of actuation is located as a white point at the top left corner in both images.

Figure 4.

Shear wave velocity data for fixed and fresh pancreatic CBs. Fixed and fresh pancreas CBs demonstrated significantly different shear wave velocities regardless if the sample was within a glass microfluidic channel, or outside on a microscope slide.

Figure 5. OCT-SWE visualization and measurement of (simulated) denser tissue within the softer surrounding CB. a) Top sample from fig. 2 was converted to a high contrast gray scale image. b and c) Corresponding cross sectional OCT images at (b) left and (c) right red vertical lines in fig. 5a (only the 4% inclusion contained microsphere scatterers). d and e) Yellow-colored 0.7 mm diameter sphere represents a 4% agarose inclusion (i.e., simulated tumor) within the 1% agarose simulated CB. In this experiment, both hydrogels contained microsphere scatterers. The sample was in d) free space and e) within a microfluidic channel. The white sloping line in e is the channel's upper wall. Shear wave speed was found to be 6.62 ± 2.65 and 11.18 ± 1.48 m/s for the background and inclusion, respectively.

Table 1

Measured density data for agarose and pancreas specimens.

SAMPLE (n = 5–10)	ρ (g/cm ³) (error < 5%)	NOTES
1% agarose	1.033	linear regression ($R^2 = 0.99$) density (g/cm ³) = 0.0136 (x% agarose) + 1.0184
2% agarose	1.046	
3% agarose	1.056	
4% agarose	1.074	
porcine pancreas (fresh)	1.017	→ 0.1% agarose equivalent
porcine pancreas (fixed)	1.026	→ 0.6% agarose equivalent
human pancreas ^{22–24}	1.079	→ 4.5% agarose equivalent