Le Bourlout, Yohann; Rehell, Minna; Kelppe, Jetta; Rautava, Jaana; Perra, Emanuele; Rantanen, Jouni; Ehnholm, Gösta; Hayward, Nick; Nyman, Kristofer; Pritzker, Kenneth P.H.; Tarkkanen, Jussi; Atula, Timo; Aro, Katri; Nieminen, Heikki J.

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Published in:
ULTRASOUND IN MEDICINE AND BIOLOGY

DOI:
10.1016/j.ultrasmedbio.2024.04.015

Published: 01/08/2024

Document Version
Publisher's PDF, also known as Version of record

Ultrasound in Medicine & Biology 50 (2024) 1247–1254

Contents lists available at ScienceDirect

Ultrasound in Medicine & Biology

journal homepage: www.elsevier.com/locate/ultrasmedbio

Original Contribution

Ultrasound-Enhanced Fine-Needle Biopsy Improves Yield in Human Epithelial and Lymphoid Tissue

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ARTICLE INFO

Keywords:
Ultrasound
Biopsy
Medical needle
Tonsil
Fine-needle aspiration
Core needle biopsy
Flexural actuation

ABSTRACT

Objective: Needle biopsy is a common technique used to obtain cell and tissue samples for diagnostics. Currently, two biopsy methods are widely used: (i) fine-needle aspiration biopsy (FNAB) and (ii) core needle biopsy (CNB). However, these methods have limitations. Recently, we developed ultrasound-enhanced fine-needle aspiration biopsy (USeFNAB), which employs a needle that flexurally oscillates at an ultrasonic frequency of ~32 kHz. The needle motion contributes to increased tissue collection while preserving cells and tissue constructs for pathological assessment. Previously, USeFNAB has been investigated only in ex vivo animal tissue. The present study was aimed at determining the feasibility of using USeFNAB in human epithelial and lymphoid tissue.
Methods: Needle biopsy samples were acquired using FNAB, CNB and USeFNAB on ex vivo human tonsils (N = 10). The tissue yield and quality were quantified by weight measurement and blinded pathologists' assessments. The biopsy methods were then compared.
Results: The results revealed sample mass increases of, on average, 2.3- and 5.4-fold with USeFNAB compared with the state-of-the-art FNAB and CNB, respectively. The quality of tissue fragments collected by USeFNAB was equivalent to that collected by the state-of-the-art methods in terms of morphology and immunohistochemical stainings made from cell blocks as judged by pathologists.
Conclusion: Our study indicates that USeFNAB is a promising method that could improve tissue yield to ensure sufficient material for ancillary histochemical and molecular studies for diagnostic pathology, thereby potentially increasing diagnostic accuracy.

Introduction

Tumors and tumor-like lesions are widely subjected to histopathological assessments to confirm a diagnosis. Due to advancements in minimally invasive procedures, needle biopsies have largely replaced the need for open biopsies in many cases (e.g., neck lumps) as a primary sample retrieval method in clinical practice [1]. With approximately 20 million procedures conducted worldwide annually, needle biopsy is cost-efficient [2] and enables rapid diagnosis (spanning from hours to a few days), thus accelerating access to potential treatments [3–6]. Moreover, the development of advanced cytological techniques and histological assessments requires increased pathological tissue; therefore, needle biopsy yield must increase to support the development of the diagnostic techniques [7–12].

The needle sample is usually obtained by (i) fine-needle aspiration biopsy (FNAB) or (ii) core needle biopsy (CNB). FNAB is a minimally invasive, rapid and inexpensive procedure for a preliminary assessment. A small hypodermic needle (commonly 21–25 G with an 0.8–0.5 mm outer diameter), typically coupled to a syringe enabling suction [13], is used. This method has a low complication rate because of the thin needle [14] and, combined with the so-called fanning technique, allows cell collection from a large volume of the target tissue [15]. However, FNAB has limitations with inadequate cell yield, from 5% to 34% for cytopathological assessments

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https://doi.org/10.1016/j.ultrasmedbio.2024.04.015
Received 17 October 2023; Revised 22 March 2024; Accepted 27 April 2024

and up to 50% for molecular assessments [7,14,16–21]. The shortcomings in yield can lead to a re-biopsy and a delay in the diagnosis, which could compromise the treatment outcome [2,7,22]. In contrast to FNAB, a larger needle (14–20 G with a 2.1–0.9 mm outer diameter) is used in CNB. With this method, a spring-loaded mechanism is often used to cut out the sample, which provides cylinderlike tissue with architecture for analysis. The technique usually provides sufficient yield for histopathological assessment, with inadequacy from 5% to 15% [1,23–27]. However, CNB requires more initial preparation and typically is associated with more complications, such as bleeding [28,29] and damage to vital organs (e.g., lung [30–32]). Therefore, CNB is not preferred for all anatomical sites [1,20,23].

To address the limitations of FNAB and CNB, we developed the ultrasound-enhanced fine-needle aspiration biopsy (USeFNAB) [33–35]. USeFNAB is aimed at collecting a greater yield of tissue fragments by actuating the needle at an ultrasonic frequency ($f \sim 32$ kHz). The movement of the needle tip is intended to produce gentle forces, sufficient to detach cells from the target tissue, resulting in an improvement of the tissue yield, while not causing excess damage to the sample. According to our previous studies, USeFNAB is able to provide up to two to three times greater tissue mass than current needle biopsy methods in ex vivo animal tissue [33]. However, the benefits of USeFNAB have not yet been demonstrated with human tissue.

In this study, we aimed to determine the improved tissue yield and identify suitable ultrasound parameters for the USeFNAB procedure, without compromising tissue sample integrity in benign human ex vivo tonsillar tissue. We chose the tonsil as a soft tissue model because of the similarity of the tissue architecture to that of lymph nodes, which are routinely sampled with needle biopsies [36]. Non-neoplastic tissue was chosen to minimize biological variation of the biopsy yield outcome, aiming to permit a more reliable assessment of the ultrasonic effects and comparison of USeFNAB against FNAB and CNB.

Methods

Biopsy devices

Three different biopsy devices were used for our experiments, as described herein.

USeFNAB

Ultrasound-enhanced fine-needle aspiration biopsy employs ultrasound to flexurally actuate the needle tip (transversal motion with respect to the needle center axis) by tens of micrometers at 32 kHz. This actuation produces shear forces, which are expected to loosen cells and tissue constructs from the target tissue. Cells and tissue constructs detached from the tissue will eventually be drawn into the needle with low pressure applied using a syringe [33]. These mechanisms are expected to increase tissue mass collection during the biopsy process. The experimental device included a Langevin transducer (custom-made mass around piezo transducer: Physik Instrumente [PI] GmbH & Co. KG, Karlsruhe, Germany, Model P-010.10H), a waveguide (3D-printed stainless steel 316L waveguide, 3D Formtech Oy, Jyvaskyla, Finland) and a hypodermic needle (21 gauge × 120 mm, Model 466564/3, 100 STERICAN, B Braun, Melsungen, Germany) (Fig. 1a). USeFNAB was developed and optimized in such a way that the ultrasonic longitudinal vibration produced by the Langevin transducer ($f \sim 32$ kHz) is converted by the waveguide to a flexural oscillation, which is amplified at the needle tip by the converging bevel geometry [35] (Fig. 1b,c). The system was controlled by a programmable function generator (Analog Discovery 2, Digilent, Inc., Austin, TX, USA), which was interfaced with custom-made MATLAB software (Release 2021B, The MathWorks, Inc., Natick, MA, USA). The function generator was connected to a custom-made amplifier (50 Ω output impedance) capable of recording the incident and reflected electrical power, thus giving, by the difference of those two signals, the consumed electrical power of the device [37]. A 5 mL syringe (Omnifix syringe 5 mL luer lock solo, B Braun) coupled with a 3D-printed custom-made low-pressure device was used to provide suction during the USeFNAB procedure (Fig. 1d). The weight of the hand piece, composed of transducer, waveguide, needle and syringe, was 69 g with simple housing. When the hand piece was coupled with the low pressure device, to allow one-hand operation, the weight was 147 g.

Fine-needle aspiration biopsy

This technique used components similar to those used in USeFNAB such as a hypodermic needle (21 gauge × 120 mm, Model 466564/3,

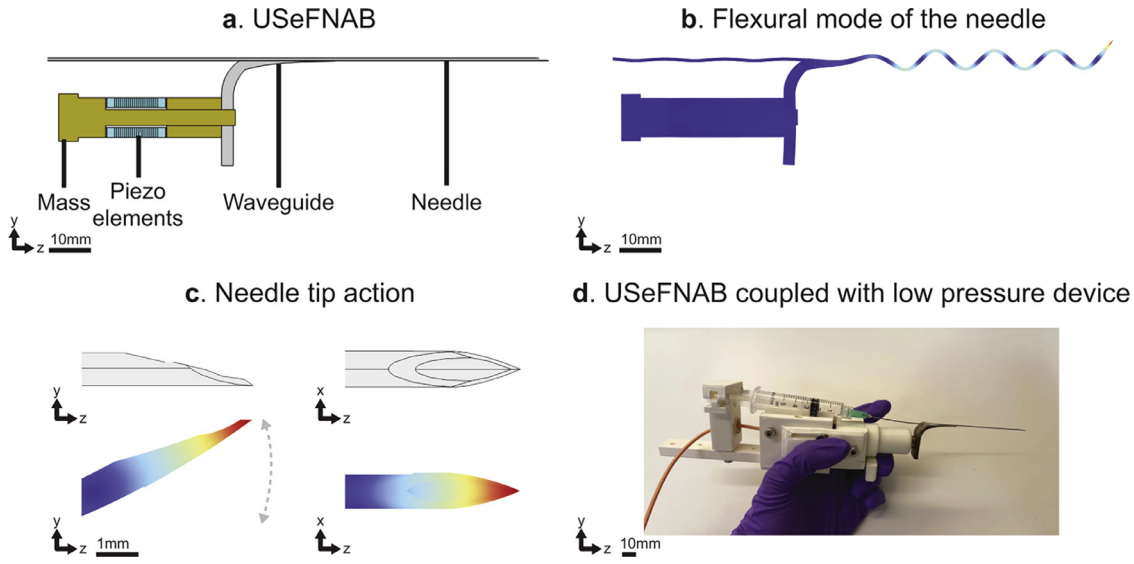

Figure 1. Visualization of the USeFNAB device. (a) Schematic of the acoustically relevant components of the USeFNAB. (b, c) Visualization of the flexural mode displacements (exaggerated) that are amplified in the waveguide and needle bevel toward the tip of the hypodermic needle. (d) Photograph of the USeFNAB device coupled with the low-pressure device and a 5 mL syringe. USeFNAB, ultrasound-enhanced fine-needle aspiration biopsy.

100 STERICAN, B Braun), which was connected to a 5 mL syringe (Omnifix syringe 5 mL luer lock solo, B Braun). The weight of the FNAB instrument was 5 g.

Core needle biopsy

The BioPince Full Core Biopsy Instrument 18 gauge was used with the throwing length of 13 mm (18 gauge, 20 cm, weight: 90 g, Model 360-2080-01, BioPince, Argon Medical Devices, Dallas, TX, USA).

Characterizing needle displacements

The purpose of this experiment was to determine the displacement of the needle tip using multiple parameters in different media (i.e., air and water) to provide a better understanding of the needle tip behavior under diverse loading conditions. To observe the needle tip displacement, a high-speed camera (model: Phantom V1612, Vision Research, Wayne, NJ, USA) in conjunction with a macro lens (model: Canon MP-E 65 mm f / 2.8 1-5x Macro Photo, Canon Inc., Ota, Tokyo, Japan) was used. The light required for the footage was provided by a broadband fiber illuminator (OSL2, Thorlabs, Newton, NJ, USA). When recording the needle tip in water, the camera was placed in front of an acrylic chamber (external dimensions = $L \times W \times H$ = 21 × 14 × 15 cm, wall thickness = 5 mm) filled with de-ionized and de-gassed water at ambient temperature (22°C). The needle tip was vertically immersed to a depth of 30 mm from the water surface. The system was driven by the custom-made amplifier coupled with the Digilent Analog Discovery 2 providing the desired signal.

Different ultrasonic parameters were investigated: duty cycle and power level. Two duty cycles were investigated: approximately 10% (60 cycles over an 18 ms period), intended to provide a greater displacement for a short period; and approximately 50% (300 cycles over an 18 ms period), intended to provide less displacement but a longer activated effect. Different power levels were investigated in correlation with the selected duty cycle, from 0.05 W (time-averaged consumed electrical power) to 0.30 W. The frequency was calibrated to be at resonance prior to the measurements ($f \approx 32–36$ kHz).

The recorded videos were analyzed in MATLAB (Release 2021B) with an algorithm capable of tracking the needle position using cross-correlation frequency technique [38] based on work of Perra et al. [33]. The maximum displacement of each footage is illustrated in Figure 2. A total of five repetitions per parameter were realized.

Biopsy and sample preparation tissue

Palatine tonsils are part of the lymphoid tissue and located on both sides of the oropharynx. They may be surgically removed in cases of recurrent acute or chronic tonsillar infections. When the indication for

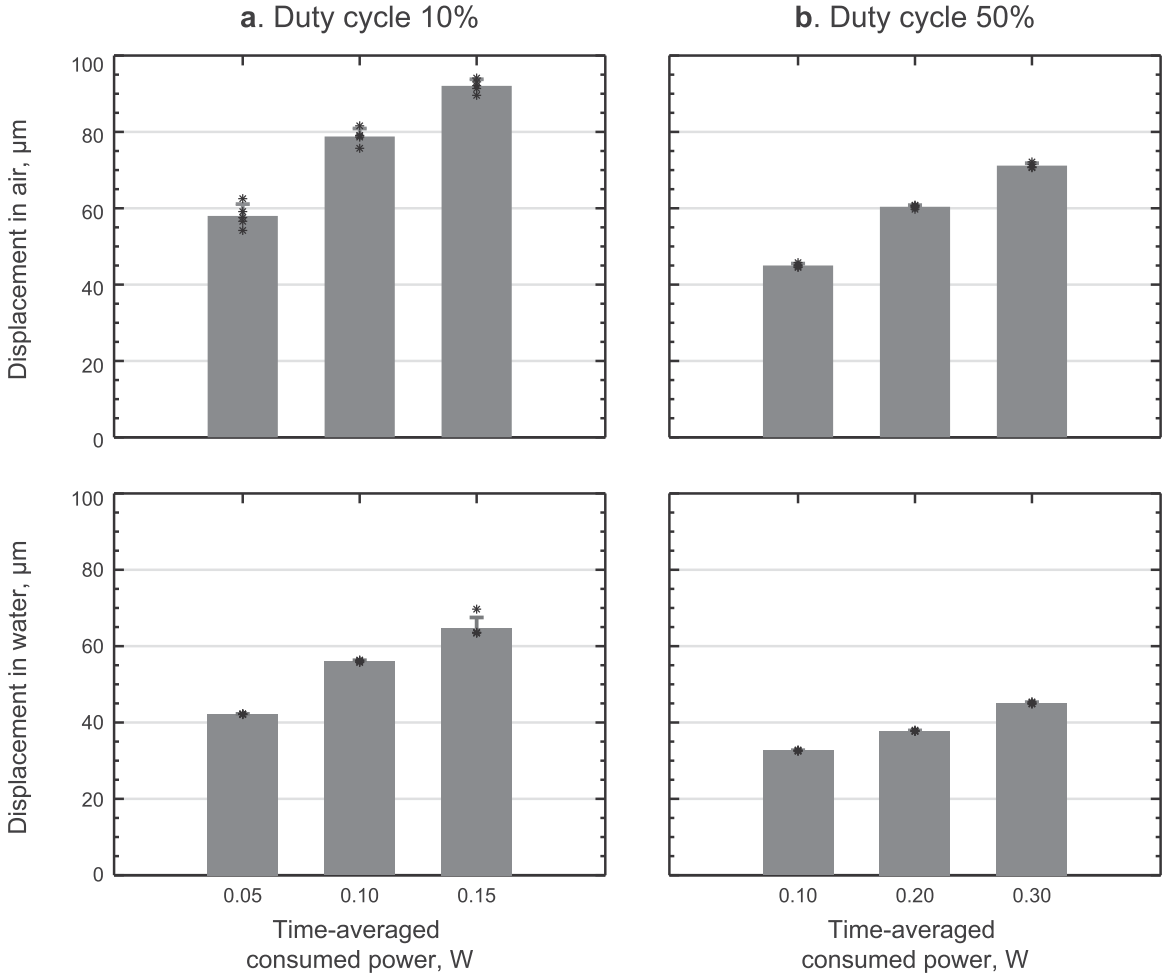

Figure 2. Flexural peak-to-peak displacements of the USeFNAB needle tip (mean ± standard deviation [SD], n = 5) in air and water using different duty cycles and power levels. The deflection of the needle tip was reduced by approximately one-third when inserted into water. When comparing the different duty cycles (a, b), an increase in deflection by one-fourth was observed with a 10% duty cycle compared with the deflection obtained with a 50% duty cycle at similar time-averaged consumed electrical power. USeFNAB, ultrasound-enhanced fine-needle aspiration biopsy.

surgery is strictly related to inflammation, the tonsils are disposed directly and do not undergo routine histopathological examination, making them easily available and with predictable and repeatable mechanical tissue properties. Moreover, a common procedure for a needle biopsy is sampling of lymph node enlargement. The enlargement may be caused by various reasons, such as metastatic lesions, inflammatory processes, lymphomas and other causes that are related to proliferation of lymphoid tissue. As tonsils consist of lymphoid tissue, it makes a great model to validate the effect of ultrasound on the improvement of the yield in relevant human tissue.

In this study, a total of 10 tonsils from 10 participants were sampled (<5 h post-excision). There were 7 women and 3 men aged 28 ± 4 y (mean ± SD, N = 10). Benign tonsil pathology was selected for our study (chronic and/or recurrent tonsillitis), and the excised tissue was required to be large enough to suit our experiment (>2 cm). The size of the tonsils varied from 2 to 5 cm in diameter.

Ultrasound parameters

Parameters similar to those described under Characterizing Needle Displacements were selected. The frequency was selected after a calibration, realized by sweeping frequencies at low power (<100 mW forward electrical power) and reading the total consumed electrical power (difference between the forward and reflected power) [37]. The frequency with the highest consumed electrical power was selected as the biopsy frequency (range of sweep: 32–36 kHz). A burst signal (repetition of activated ultrasonic signal followed by inactivated ultrasonic signal) with a pulse repetition frequency of 55 Hz was used during the biopsies. This allowed adequate control of the ultrasonic needle action. The different duty cycles and power levels disclosed previously were investigated.

Biopsy protocol

Biopsies were performed 2–5 h post-excision at room temperature (22°C–24°C). The resection was immobilized on styrofoam with five sewing needles and covered with moist cotton gauze between the biopsies to prevent drying.

The USeFNAB procedure consisted of the insertion of 1–2 cm of the needle tip into the tissue, followed by a calibration of the device. Low pressure was applied by pulling the plunger back to the 3 mL mark of the syringe, and the ultrasound was turned on for 10 s. A fanning [39] with a penetration depth of approximately 0.5–1 cm, stroke frequency of 1 Hz and approximate angle of 7° between the stokes was used. After the biopsy duration, the low pressure was released before extraction of the needle from the tissue. The needle and waveguide were weighed pre- and post-biopsy to measure the mass of the harvested sample. Following this, the sample was expressed directly into formalin by applying positive pressure with the syringe plunger.

For FNAB, we used a procedure similar to USeFNAB, but without the ultrasonic sequence.

The CNB device was loaded and set to the 13 mm displacement. The needle was then inserted by 0.5–2 cm inside the tissue. After deployment, the needle was removed from the tissue and the core biopsy sample obtained was weighed. This core was then immersed in formalin.

A single needle dwell was used for the different biopsy techniques. All biopsies were realized by the same user who has been trained by radiologists and medical doctors prior to the experiments.

Sample fixation

All samples obtained were processed at the Department of Pathology, HUS Diagnostic Center and HUS Helsinki University Hospital (Helsinki, Finland) according to standard protocols. The samples were fixed with ethanol (70%), dehydrated, cleared with xylene, waxed, embedded in paraffin, sectioned (slice thickness 3–4 μm) and stained with hematoxylin and eosin (H&E). The immunohistochemical stainings were performed according to standard diagnostic protocols with the Ventana Benchmark ULTRA Immunoautomat (Ventana Medical Systems, Inc., Oro Valley, AZ, USA). The following antibodies were used: AE1/AE3 (CK-PAN), clone AE1/AE3/PCK26 (Catalog No. 790-2595) with ready-to-use dilution (Ventana Medical Systems, Inc.), pre-treatment CC1 for 64 min and incubation time for primary antibody 32 min; CD45 (LCA), clone 2B11 + PD7/26 (Catalog No. 760-4279) with a ready-to-use dilution (Roche Holding AG, Basel, Switzerland); pre-treatment CC1 for 36 min, incubation of primary antibody 20 min; Vimentin, clone V9, (Catalog No. M0725) with 1:1000 dilutions (Agilent Technologies, Inc., previously Dako, Santa Clara, CA, USA), pre-treatment CC1 for 64 min and incubation of primary antibody for 44 min. Detection with all antibodies was visualized with DAB (UltraView DAB, Ventana Medical Systems, Inc.).

Histopathological assessment sample quantity

Two sample quantity measurements were conducted: (i) weight measurement and (ii) microscopic evaluation. Weight measurement was conducted immediately after the biopsy. The extracted sample was weighted using a precision scale (ADJ 200-4, mass range: 400 mg –210 g, KERN & SOHN GmbH, Balingen, Germany). The microscopic evaluation of sample quantity was conducted visually by the two pathologists, both blinded to the biopsy modality. A five-level scale was employed: 0 = empty slide; 1 = very small sample, inadequate for diagnosis (single cells or small cell clusters); 2 = small sample (cell clusters and tissue fragments), but adequate for diagnosis; 3 = moderate sample (several tissue fragments, adequate); 4 = excellent sample (abundant cells and tissue fragments, adequate). After individual assessments, if a discrepancy between the two pathologists was observed, joint re-evaluation of the sample was conducted to reach to a convergent view of the assessment.

Sample quality

Microscopic morphological tissue quality was evaluated, in a blinded manner, by two pathologists on a four-level scale: 1 = 0–25% of the tissue were preserved in good quality, 2 = 26%–50%, 3 = 51%–75% and 4 = 76%–100% of good quality preservation. Signs of coagulation and fragmentation lowered the quality score. After individual assessments, if a discrepancy between the two pathologists was observed, joint re-evaluation of the sample was conducted to reach a convergent view of the assessment.

Immunohistochemical staining analysis

Two immunohistochemical stainings were tested in this study. (i) CKPAN was used as it has immunoreactivity in epithelia. The tonsillar lymphoid tissue is layered by squamous epithelium and has crypt structures that demonstrate positivity with this stain. Moreover, CKPAN is a reliable stain for cytological samples for diagnostic assessments. (ii) The other immunohistochemical staining selected, CD45, is used by pathologists to highlight inflammatory cells or determine the hematopoietic nature of tumors of unknown origin with high specificity. As the tonsillar lymphoid tissue is composed of lymphoid cells, this staining provides a good control to measure cell antigen persistence. Immunohistochemical stainings were analyzed by pathologists, who then compared the results obtained with the routine positive control. Accuracy of the staining, as well as the staining quality and intensity, was compared.

Statistical test

A non-parametric statistical test, the Wilcoxon signed-rank test (α = 0.05, two-tailed) coupled with the Bonferroni correction (statistical significance level was 0.0071), was used to compare USeFNAB with

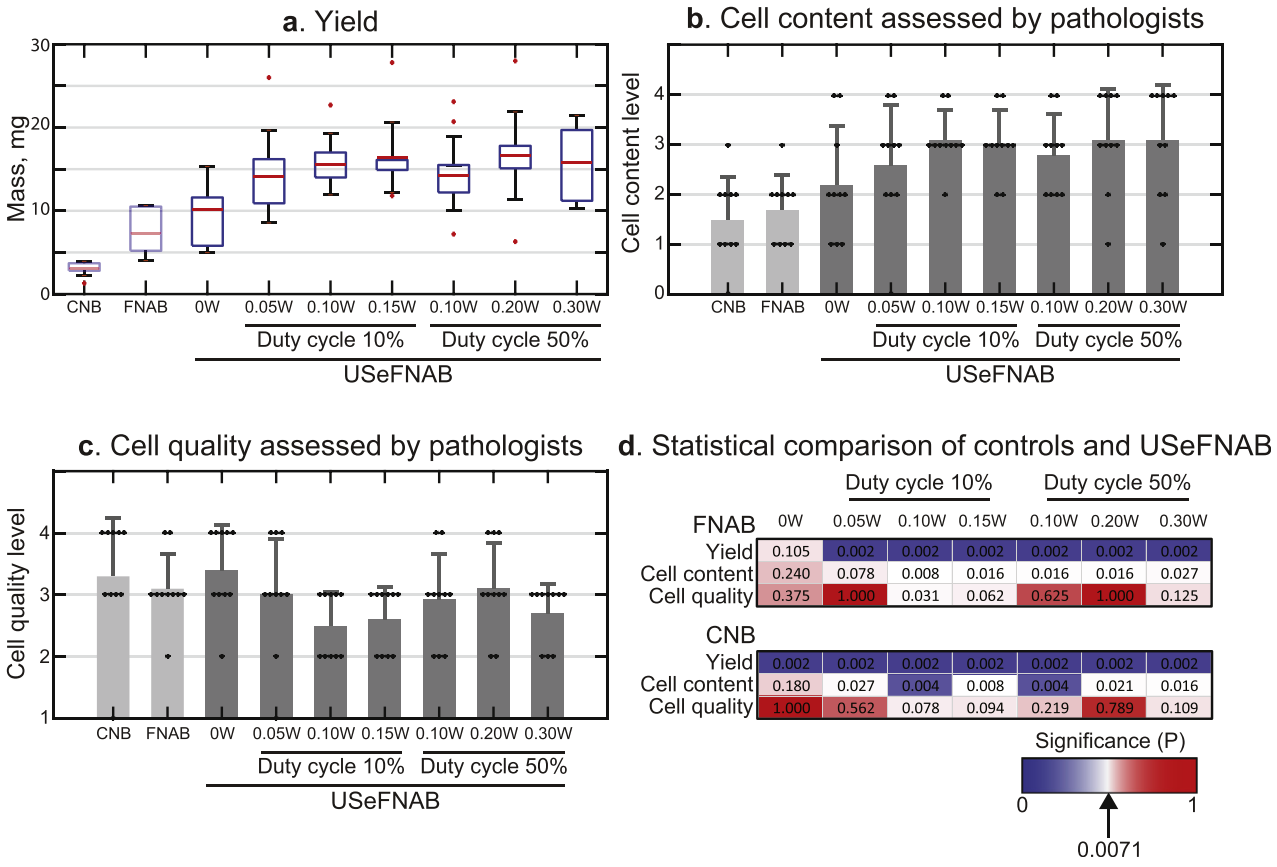

Figure 3. Measurements and assessments of the biopsy results. (a) Tissue yield was defined as the mass of collected tissue samples, obtained with different biopsy methods (the central mark represents the mean, the box represents the 25th to 75th percentile and the error bars represent the standard deviation, outliers are represented by points, n = 10). Powered USeFNAB appears to collect more tissue than the other methods, and the mass collection increases with the electrical power consumed (p = 0.002). (b) Assessment of cell content observed by the pathologists under microscopy (bars represent the means, error bars represent standard deviations, n = 10). The quality scale ranges from 0 to 4, where 0 is an empty slide with no sample and 4 is a diagnostically considerable amount of tissue (≥2 being adequate for diagnosis). (c) Cell quality assessed by the pathologists (mean ± standard deviation, n = 10). The scale ranges from 1 to 4, where 1 refers to a damaged sample (0–25% of the sample remained intact) and 4 refers to a sample in excellent condition (75%–100% of the sample remained intact). (d) Statistical comparison of controls and USeFNAB using the Wilcoxon signed-rank test (α = 0.05) coupled with the Bonferroni correction (statistical significance level = 0.0071). The *color bar* indicates visually the proximity of the tested value from statistical significance. *Blue* indicates that statistical significance was reached, *white* indicates a value close to statistical significance and *red* indicates no statistical difference. The results suggested improvement of tissue yield with various ultrasound power levels. Although at 10% and 50% duty cycles, the sample quality with USeFNAB did not statistically significantly differ from that with FNAB, at 0.05 W for 10% and at 0.1–0.2 W for 50% duty cycles, respectively, sample quality was on average comparable to that of FNAB. CNB, core needle biopsy; USeFNAB, ultrasound-enhanced fine-needle aspiration biopsy.

FNAB and CNB. The statistical analysis was realized in MATLAB (Release 2021B).

Ethics statement

The study was conducted in accordance with national regulations and institutional guidelines. The research ethics committee of Helsinki University Hospital approved the study, and institutional permission was granted (study permit: HUS/241/2021). Informed consent was obtained from all participants and/or their legal guardian(s).

Results

Quantification of the needle tip activity

The needle tip displacement of USeFNAB at resonance ($f \approx$ 33.4 −35.8 kHz) was optically quantified. At 10% duty cycle in air, the peak-to-peak needle tip displacement varied from 58.0 ± 3.1 μm (mean ± SD, n = 5) to 92.1 ± 1.7 μm (n = 5) at time-averaged consumed electrical powers of 0.05 and 0.15 W, respectively (Fig. 2a). At 50% duty cycle in air, the needle tip movements were smaller, that is, from 45.0 ± 0.5 to 71.1 ± 0.7 μm (n = 5 per group) at powers of 0.10 and 0.30 W, respectively (Fig. 2b). When the needle was mechanically loaded by immersing its tip in water, the displacements were reduced compared with the unloaded situation, that is, 42.2 ± 0.1 to 64.7 ± 2.8 μm and 32.5 ± 0.3 to 45.1 ± 0.3 μm (n = 5 per group) for 10% and 50% duty cycles, respectively, within the tested power range.

Quantification of retrieved tissue yield

Retrieved tissue yield was quantified from samples biopsied from the ex vivo human tonsils. The masses obtained measured were 7.3 ± 3.3 mg (mean ± SD, n = 10) with FNAB and 3.1 ± 0.9 mg (n = 10) with CNB. USeFNAB provided yields of 16.4 ± 4.2 mg (n = 10 per group) at 0.15 W with a 10% duty cycle and 15.9 ± 5.6 mg at 0.30 W with a 50% duty cycle (Fig. 3a). A minor average increase in yield was observed when using USeFNAB at 0 W consumed electrical power (10.2 ± 5.2 mg) compared to FNAB, but this was not statistically significant (p = 0.105). However, when USeFNAB was powered, a consistent and statistically significant yield enhancement was observed as compared with FNAB or CNB (Fig. 3d) (p = 0.002; statistical significance level obtained from the Wilcoxon signed-rank test [α = 5%] with the Bonferroni correction was 0.0071).

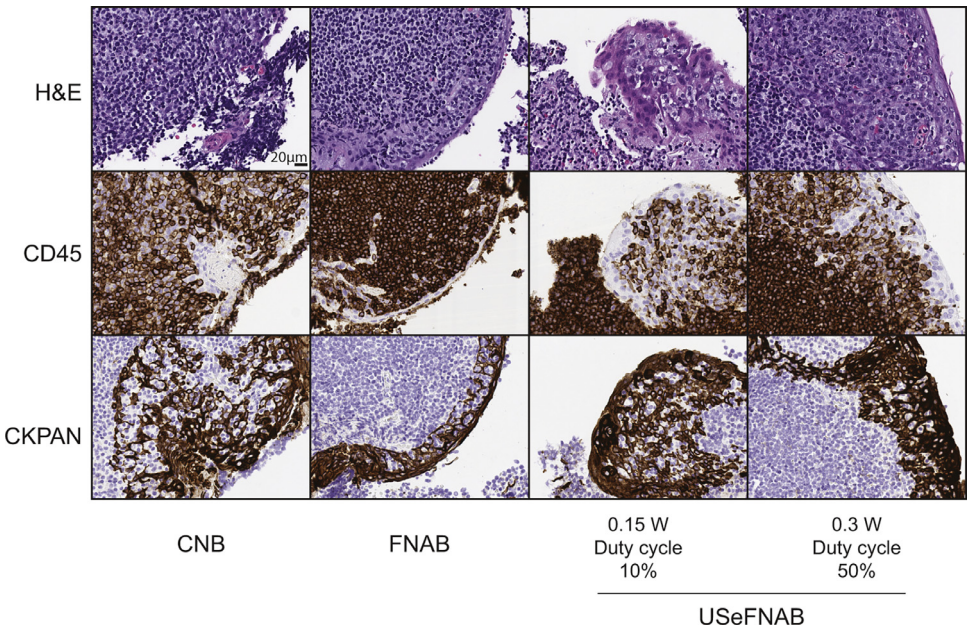

Figure 4. Microscopy images of representative histological samples obtained by using the different sampling techniques on human ex vivo tonsil (scale bar = 20 μm in the top left image). The results revealed that intact epithelial and lymphoid cells were present in each technique, and no differences in stainings were recorded across the groups. The results also reveal that the ultrasound technique affected neither the staining quality (hematoxylin and eosin, CD45 and CKPAN) nor the immunoexpression of the tested stainings in histological samples (CD45 and CKPAN). CNB, core needle biopsy; USeFNAB, ultrasound-enhanced fine-needle aspiration biopsy.

Histologically estimated tissue yield

The tissue obtained from the biopsies was analyzed from histological sections independently by two pathologists, both blinded to the biopsy method. Pathologists graded the sample quantity by visual assessment on a scale from 0 to 4 (0 = empty sample, 4 = considerable amount of tissue), with 2 representing adequate tissue. The trend in histological assessment of yield was in line with sample mass (Fig. 3a,b) (Spearman's correlation coefficient [r_S] of the means = 0.9154, two-tailed). CNB and FNAB received scores of 1.5 ± 0.9 (mean ± SD, n = 10) and 1.7 ± 0.7 (n = 10), respectively. USeFNAB at 0 W scored 2.2 ± 1.1 (n = 10). With a 10% duty cycle at 0.15 W, the cell content was 3.0 ± 0.7 (n = 10), while with a 50% duty cycle at 0.30 W, the pathologists assessed the grade as 3.1 ± 1.1 (n = 10).

Histologically estimated sample quality

As described under Histologically Estimated Tissue Yield, the pathologists evaluated the quality of the sample on a scale of 1–4. The scale allowed visual estimation of the amount of intact sample (1 = 0–25% and 4 = 76%–100% of good quality preservation). USeFNAB at 0 W, CNB and FNAB provided the highest quality grades at 3.4 ± 0.7, 3.3 ± 1.0 and 3.1 ± 0.6 (mean ± SD, n = 10), respectively (Fig. 3c). The lowest quality, 2.5 ± 0.5 (n = 10), was obtained with the 10% duty cycle at 0.10 W consumed electrical power. At equivalent power, the 50% duty cycle, exhibited a cell quality level of 2.9 ± 0.7 (n = 10).

Figure 4 illustrates the results of the H&E staining and two immunohistochemical staining techniques (CD45 and CKPAN). All immunohistochemically stained samples were comparable with each other and to the routine positive controls. The use of ultrasound during the biopsy affected neither the staining quality nor the staining intensity, when compared with other biopsy methods.

Discussion

Needle biopsy is typically an easy-to-use and rapid procedure for obtaining a tissue sample for diagnostic purposes. However, the collected material may not always be sufficient, so the diagnosis may be inconclusive. In the study described here, we determined that the novel experimental biopsy method USeFNAB provides an increased quantity of tissue for pathological diagnostics without significantly compromising the sample quality.

In epithelial and lymphoid tissue, powered USeFNAB resulted in consistent and statically significant increases in mass, up to 2.2- and 5.3-fold, compared with the masses obtained with FNAB and CNB, respectively (p = 0.002). The cell content assessment, carried out blindly by pathologists using visual microscopic evaluation, revealed a similar trend compared with the yield measured by weighing (Spearman's correlation coefficient r_S = 0.9154). The cell content assessed by pathologists did not statistically significantly differ between powered USeFNAB and FNAB or CNB ($0.008 \leq p \leq 0.078$), except at the power level of 0.10 W (p = 0.004, Fig. 3d). However, an increase in the average values was observed.

The cell quality assessed by the pathologists did not statistically significantly differ ($p \geq 0.031$) in the USeFNAB group compared with the FNAB and CNB groups. Although a decreasing trend in cell quality level was seen with increasing total consumed electrical power, the quality with the 50% duty cycle was better maintained. The tissue collection improvement, as well as the damage observed from the results, may rise from the needle tip displacement. A smaller deflection was observed once the needle tip was loaded (i.e., in water), but independent of the load, the increase in the total electrical power consumed was seen to translate into an increase in the needle tip displacement (Fig. 2). Similarly, duty cycle affected needle tip deflection, whereby the smallest duty cycle provided the greatest displacement for a given time-averaged power. This is because by smaller ultrasonic activation times led to greater instantaneous electrical power, while time-averaged power remained constant, explaining the greater yield and slightly greater damage to the sample using high power and small duty cycle observed in our experiments. Therefore, a lower duty cycle may not be preferred in all situations such as biopsy of epithelial and lymphoid tissue from the perspective of maintaining sample quality.

Immunohistochemical staining and molecular and genetic diagnostic methods are and will be increasingly important tools in cancer diagnostics [40]. In this study, we tested three key staining methods to rule out ultrasound-induced effects on staining quality. On the basis of the

assessment by pathologists, the immunohistologic stainings were not found to be influenced by ultrasound energy. These results indicate that within the limitations of this study, ultrasound does not appear to influence conventional histological and immunohistochemical stainings. On the contrary, USeFNAB provided more tissue, and is expected to be useful for applications in which insufficient quantities of pathological cells are frequently obtained [7].

There are a few limitations to this study. The design of USeFNAB, which is slightly larger than FNAB, may change the use of the needle and have a minor effect on the sampling. Blood flow was not present, and the tissue temperature (~22°C) differed from a physiologically relevant temperature. Although the time from excision to sampling was minimized (<5 h), autolytic effects could occur without being clearly detected by histology. Moreover, the sample size is limited, and it is probable that different tissues might react differently to similar ultrasound exposure. Thus, further studies are required to reveal the effect of ultrasound on pathological human tissues, with greater sample size and under in vivo conditions.

Conclusion

This study is the first demonstration of the feasibility of using USeFNAB with ex vivo human epithelial and lymphoid tissue. The results suggest that, with adequate parameters, ultrasonic actuation improves fine-needle biopsy yield while maintaining sample quality in ex vivo human tissue. This potentially opens avenues for auxiliary tests, such as cytochemical and genomic studies, thus enabling more precise diagnostics through improved tissue yields.

Conflict of interest

H.J.N., K.P.H.P. and Y.L.B. have roles and stock ownership in Swan Cytologics Inc., Toronto, ON, Canada. H.J.N. and K.P.H.P. are inventors within the patent family associated with WO2018000102A1. Y.L.B., G. E. and H.J.N. are inventors within the patent family associated with WO2020240084A1. Y.L.B., G.E. and J.R. are inventors with the patent family associated with EP4101402A1. Remaining authors have no competing interest to declare.

Acknowledgments

Business Finland (Grant 5607/31/2018) and the Academy of Finland (Grants 314286, 311586 and 335799) are acknowledged for financial support. We thank the Helsinki University Central Hospital (HUS) Pathology Department for their facilities and equipment. We also thank the Department of Otorhinolaryngology—Head and Neck Surgery, HUS, for providing the tissue samples.

Data availability statement

The curated data and the codes are available on reasonable request to the corresponding author (Heikki J. Nieminen, heikki.j.nieminen@aalto.fi).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ultrasmedbio.2024.04.015.

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