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Application of ultrasound in preparing pathological sections to reduce processing time

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Abstract

It has been found experimentally that application of sub-mega and low megahertz ultrasound (US) of spatial and temporal averaged intensity I_{sata} up to 10 W/cm^2 during the process of preparing pathological sections of the mouse tissue has shortened the processing time from 12 h (without US) to less than half an hour (with US). The experiment has also showed that the processing time reached the shortest for ultrasound $f = 200$ kHz among the frequencies of 200 kHz, 400 kHz, 600 kHz, 800 kHz and 1 MHz used in this study. It has been proposed that ultrasound inducing non-inertial cavitation enhanced the permeability of cell membrane to liquid. Thus tissue fixation and dehydration were speeded up by application of US.

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Keywords: Ultrasound; Tissue processing; Cell membrane permeability; Pathological sections

1. Introduction

Pathologists heavily rely on tissue specimens to prepare slides for examinations and analysis. The conventional tissue processing (CTP) as a gold standard of preparing a specimen of pathological sections has existed for about 100 years [1]; it includes the following steps: (1) tissue fixation; (2) dehydration; (3) transparence (making the specimen optically transparent); and (4) embedding (infusing of liquid-state paraffin). The whole processing time for one pathological section is approximately 12 h [2]. The main weakness of CTP is it is time-consuming and usually introduces at least one day delay for medical personnel to be able to observe a slide of pathological specimen after a biopsy is performed. It is always desirable to search a new processing technology to speed up this process.

The ultrasound-mediated rapid tissue processing (URTP) method has been developed in our laboratory recently and was applied to the preparation of the pathological section of mouse tissues. The quality of pathological slides prepared from the pathological section generated using the URTP method was examined by pathologists and considered to be just as good as those prepared from the CTP method. It has been shown that the URTP processing time in preparing a mouse tissue specimen can be shortened to less than half an hour, that is much less than that of the CTP method. It was also observed that the processing time of the URTP method was ultrasound frequency-dependent; for the same kind of tissue specimens, the preparation time could be different with different ultrasound frequencies. Among the group of 200 kHz, 400 kHz, 600 kHz, 800 kHz and 1 MHz applied, 200 kHz ultrasound provided the shortest processing time.

In this article, the experimental method including sample preparation and handling and experimental data are

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presented; the possible physical mechanism involved with this technology was proposed.

2. Method

2.1. Specimens

Mouse tissue specimens prepared from kidneys of mice (ICR series, 3 month of age and 30 g of weight) were prepared by medical personnel at the Gu-Lou Hospital, affiliated to the College of Medicine, Nanjing University according to a protocol approved by the Internal Review Board. The dimensions of specimens were equal to 5 mm × 5 mm × 2 mm. Efforts were made to keep the tissues as fresh as possible; the goal was to keep the original structure at the cellular level of the specimens unaltered.

2.2. Handling

Prepared tissue specimens of a mouse kidney were put into a series of four glass beakers filled with various reagents corresponding to each step as shown in Fig. 1. The whole tissue processing course for tissue specimens consisted of the following steps (Fig. 1): (1) fixation with pure formaldehyde; (2) dehydration with 95% alcohol; (3) transparence with pure xylene; (4) infusing heated liquid-state paraffin into the glass beaker. The ultrasound was applied on the tissues during the whole process (see description below).

2.3. Ultrasound exposure

As shown in Fig. 2, a portion of the bottom wall of a beaker, whose size was as big as the size of an ultrasound transducer, was cut to allow the ultrasound transducer mounted water-tightly at the bottom of the beaker with glue. The transducer faced upward aiming those specimens in the beaker filled with various reagents. All devices were placed into a constant temperature water bath of dimensions of 25 cm × 16 cm × 10 cm. The temperature of the water bath was kept at 60 °C. The ultrasound intensity and frequency are controlled by an ultrasound signal generator (home-made). The adjustable frequency range was between 100 kHz and 2 MHz, the acoustic intensity I_{sata} (spatial averaged and temporal averaged intensity) was

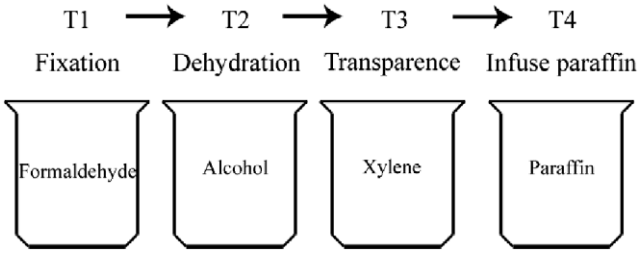

Fig. 1. Illustration of the ultrasound-mediated method for rapid tissue processing.

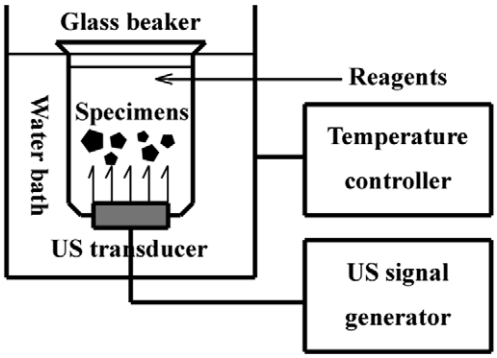

Fig. 2. The experimental setup.

controlled under 10 W/cm^2 measured by the acoustic radiation method [7].

3. Results

3.1. Processing time

Our experiments have shown that the whole processing time is a function of ultrasound frequency; the total processing time was less than half an hour for all frequencies tested. The minimum processing time of each step was achieved by comparing the quality of the slide made from the tissue section with that made from the CPT method. When the quality of a pair of slides was indistinguishable to us, we considered we reached our goal and the time spent in the processing was registered as the minimum processing time of this step. As an example, Fig. 3 shows that six blocks of specimens prepared from a piece of kidney tissue from the mouse; they were processed with the following five exposure US frequencies; they were 200 kHz, 400 kHz, 600 kHz, 800 kHz and 1.0 MHz respectively. Slides prepared from these specimens were compared with a control shown in Fig. 3f processed using the conventional tissue processing method (no US). A summary of processing time of the URTP method corresponding to US frequencies of 200 kHz, 400 kHz, 600 kHz, 800 kHz and 1.0 MHz is

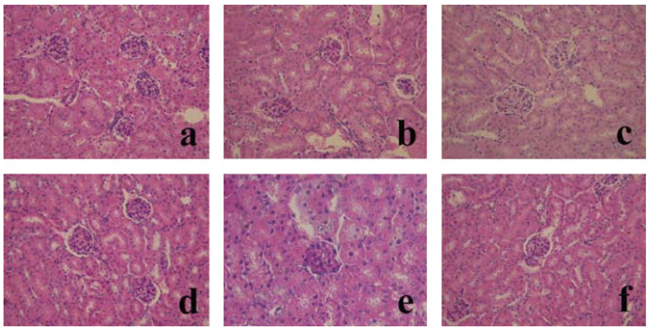

Fig. 3. Images (200×) of panels (a)–(e) are taken from slides prepared from five mouse kidney sections with US. Their frequencies are respectively (a) 200 kHz, (b) 400 kHz, (c) 600 kHz, (d) 800 kHz, (e) 1.0 MHz. (f) Image of a mouse kidney section prepared using CPT method (no US).

Table 1
Comparison of the processing time (mean ± standard deviation) of various US frequencies 200, 400, 600, 800 and 1000 kHz

Frequency (kHz)	Fixation (s)	Dehydration (s)	Transparence (s)	Dip-wax (s)	Total time (s)
200	240 ± 7.4	181.2 ± 6.4	239.7 ± 9.8	358.7 ± 14.3	1019.8 ± 18.8
400	299.3 ± 14.7	359.7 ± 8.0	299.7 ± 14.7	422.5 ± 14.2	1381.2 ± 33.3
600	361.3 ± 10.2	421 ± 8.2	302.3 ± 15.7	421.8 ± 16.3	1506.5 ± 24.7
800	238.8 ± 7.6	240.5 ± 4.8	242.7 ± 8.2	360.7 ± 11.4	1082.7 ± 16.6
1000	361.7 ± 19.7	419.5 ± 10.0	300.8 ± 16.4	480.2 ± 16.7	1562.2 ± 27.7

Table 2
The evaluation results of comparing the quality of 100 pairs of pathological mouse kidney tissue slides respectively prepared by URTP method and CTP methods by five pathologists

Pathologist no.	Indistinguishable	Rapid-section is better	Conventional-section is better
1	53	25	22
2	50	27	23
3	60	19	21
4	62	21	17
5	51	27	22
Total no. (%)	281 (55.2%)	119 (23.8%)	105 (21%)

shown in Table 1. Each processing time is presented as the mean of 30 specimens ± standard deviation.

3.2. Tissue slide quality comparison

Total of 200 slides were prepared from 200 mouse kidney tissue sections described above. Among them, 100 were prepared using the URTP method (with US) and the other 100 were prepared using the CTP method (no US). They were paired (one URTP method treated and the other CTP method treated) in random. Each pair was examined by pathologists without prior knowledge of the nature how the slide was prepared. Pathologists were asked to rate the quality of slides using grades of 1–3 (1 is the best, 2 the worst and 3 indistinguishable). Five pathologists with various years of working experience participated evaluations.

The double-blind-evaluation results are summarized in Table 2. Among the 100 pairs, 55.2% pairs were rated as indistinguishable, 23.8% pairs were rated as URTP method treated ones are better and 21% pairs were rated as CTP method treated ones are better. The results summarized in Table 2 suggested that no distinguishable difference in quality between URTP and CTP method treated slides.

4. Conclusions and discussion

In conclusion, the URTP method has been applied in preparation of the pathological sections of the mouse tissue. The URTP method has significantly reduced the processing time from 12 h to half an hour and meanwhile kept the quality of the prepared tissue section undistinguishable from that of CTP treated sections. During the ultrasound assistant processing, abundant small actively moving bubbles were observed near the tissue samples. In our opinion, the role of ultrasound in this study is similar to that in sonoporation [3–6], i.e., ultrasound inducing non-inertial (stable) cavitation enhanced the permeability of cell membrane to liquid. Thus tissue fixation and dehydration were speeded up by application of US. It is possible that bubble oscillations near tissue samples may generate shear stress on the cell membranes [8]. It in turn enhanced cell permeability, a similar process happened during sonoporation [3–6].

Mechanical index MI has been used to describe the possible bioeffects associated with cavitation (microbubbles' activities) induced by diagnostic ultrasound imaging systems; it is defined by $MI = p_{-}/\sqrt{f}$ [9], where p_{-} in MPa and f in MHz are in situ rarefaction amplitude after attenuation correction due to tissue and frequency of ultrasound respectively. Mechanical index (MI) was not defined for our application. But, we might use it to measure the likelihood of bubbles' activity stimulated by ultrasound. It is suggested by MI that low frequency may have high probability to induce cavitation; it seems to agree with the result that the lowest frequency (200 kHz) among the group of frequencies used has the best result. Additionally, it is also plausible that vigorous tissue specimen mixing effect by ultrasound may also play a role in the process by enhancing mass transport and convection in the sample. However, the fact that the lowest frequency (200 kHz) has maximum effects seems not to favor this explanation.

Further experiment is needed to fully understand the physical mechanism of this technique.

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References

[1] G. Arendt, Apparat zur selsstatigen Fixierung und Einbettung mikroskopischer praparate, Munchen. Med. Wochen. 56 (1909) 2226–2227.

[2] A.R. Morales, M. Nassiri, R. Kanhoush, V. Vincek, M. Nadji, Experience with an automated microwave-assisted rapid tissue processing method – validation of histologic quality and impact on the

timeliness of diagnostic surgical pathology, Am. J. Clin. Pathol. 121 (4) (2004) 528–536.
[3] S. Bao, B.D. Thrall, D. Miller, Transfection of a reporter plasmid into cultured cells by sonoporation in vitro, Ultrasound Med. Biol. 23 (1997) 953–959.
[4] D.L. Miller, S. Bao, J.E. Morris, Sonoporation of cultured cells in the rotating tube exposure system, Ultrasound Med. Biol. 25 (1998) 143–149.
[5] M. Ward, J. Wu, J.F. Chiu, Experimental study of the effects of Optison® concentration on sonoporation in vitro, Ultrasound Med. Biol. 26 (2000) 1169–1175.
[6] J. Wu, Sonoporation, gene transfection, anticancer drug and antibody delivery using ultrasound, in: Junru Wu, Wesley Nyborg (Eds.), Emerging Therapeutic Ultrasound, World Scientific, New Jersey, 2006.
[7] K. Beissner, Measurement techniques, in: M.C. Ziskin, P.A. Lewin (Eds.), Ultrasound Exposimetry, CRC, Boca Raton, 1993.
[8] J. Wu, Shear stress in cells generated by ultrasound, Prog. Biophys. Mol. Bio. 93 (2006) 363–373.
[9] NCRP, Exposure criteria for medical diagnostic ultrasound: II. criteria based on all known mechanism, National Council on Radiation Protection and Measurements, Betheda, MD, 2002.