ULTRASOUND AFFILIATED LOW TEMPERATURE FORMALIN FIXATION

--- A MOLECULAR FRIENDLY FORMALIN FIXATION METHOD

Haiqi He[1], Hanxiang Zou[1], Wei Sing Chu[2]

1. Bio-Quick Corp, Rockville, MD 20852 2. Department of Scientific Laboratories, Armed Forces Institute of Pathology, Washington, DC 20306

Abstract

With the rapid development of molecular assays and the onset of personalized medicine, clinical diagnoses of cancer and other diseases will rely increasingly on macromolecules from preserved tissue specimens. However, the formalin-fixing and paraffin-embedding (FFPE) tissue preservation method, as the most commonly used approach for modern histology, does not properly preserve the biomarkers needed for these diagnoses. In this paper, we demonstrated that tissues fixed in formalin at a refrigerated temperature (4°C–10°C) generated better morphology than those fixed by the conventional room temperature method. Biomolecules in the tissues fixed at refrigerated temperature were also better for molecular assays. We further demonstrated that ultrasound (US) irradiation could significantly reduce time needed in fixing tissues in formalin at a cold temperature. Our study provides strong evidence that much-improved FFPE tissue samples that are suitable for both the histological diagnosis and molecular analysis can be produced by combining ultrasound with low temperature in the formalin fixation step.

Methods

The US waves were generated by a prototype US device constructed by the Bio-Quick Corporation (Rockville, MD). US signals at a frequency of about 1.2 MHz and powers up to 100 W were applied to solutions located in a US chamber. The US chamber is a double-wall cylinder with a 3-inch diameter piezoelectric transducer sealed on the bottom of the chamber (as seen in Fig. 1). RCB 300 refrigerated circulating bath (Hoefer Scientific Instrument, San Francisco) was connected to the US chamber for maintaining the reagent to a preset temperature (4°C-25°C) and remove the heat generated by US irradiation.

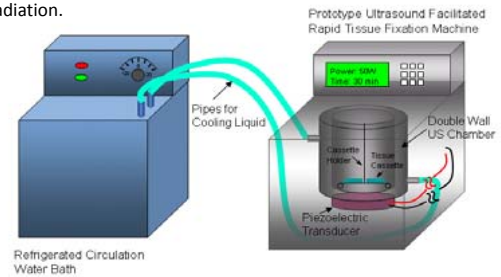

Fig. 1 Schematic diagram of experimental setup for low temperature ultrasound (US)-facilitated formalin fixation and paraffin embedding (LT-US-FFPE)

Results

Tissues fixed at 4 °C in formalin overnight generated brighter and sharper edge as well as better sub-cellular details than those fixed at room temperature. Quantitative analysis on number of nuclei per field and nuclei size did not show any cellular or intracellular shrinkage due to low temperature.

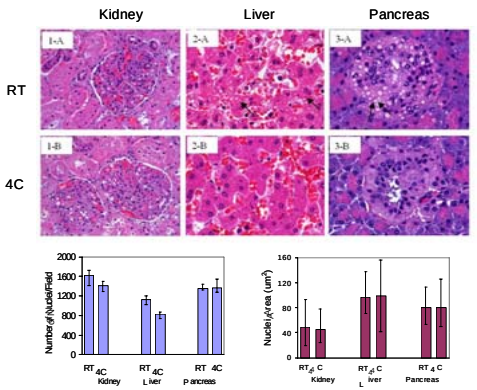

Figure 2. Comparison of H&E staining of bovine kidney (1), liver (2), and pancreas (3) tissues fixed at (A) room temperature overnight and (B) 4ºC (upper panel); and the quantification of number of nuclei per field as well as nuclear size (lower panel).

Ultrasound significantly reduced time required for tissues to be fixed in cold formalin from 18-24 hours to half an hour.

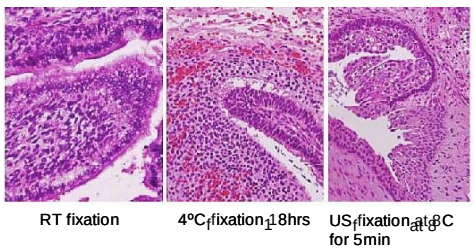

Figure 3. H&E staining results of ovary cancer tissues fixed in formalin at RT and 4° C for overnight and under US at 8 °C for min, respectively

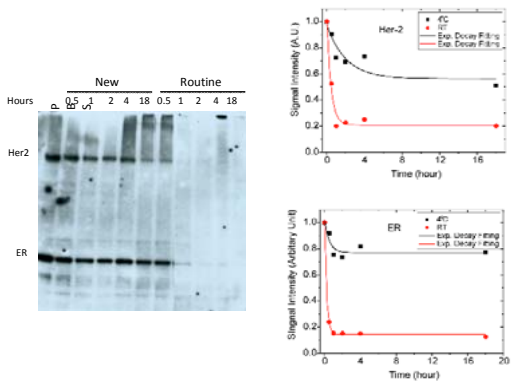

Figure 4. Western blot analysis on lysates of T47D cells fixed in neutral buffered formalin at 4 C or RT for various lengths of time. Left: Western blot membrane probed with pooled antibodies against HER-2 and ER. Right: Plots for digital intensities of HER-2 and ER Western blot signals against fixation time.

Tissues preserved by US facilitated low temperature fixation produced proteins more available for IHC and Western blot assays. US-LT-FFPE reduces the masking of protein antigenicity. Antigen retrieval (AR) is not required in IHC staining for many antibodies that otherwise require AR on routine FFPE tissue section.

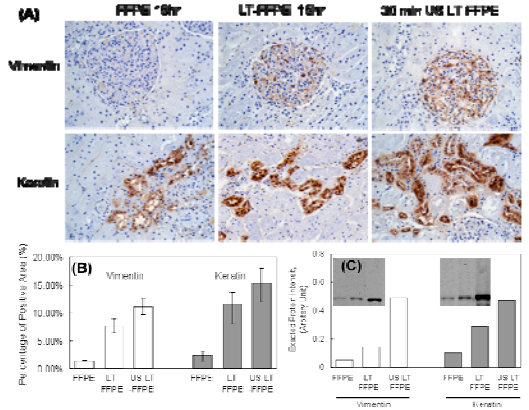

Fig. 5 IHC and Western blot studies on cow kidney tissue fixed for 30 min at 4°C with US irradiation (US-LT-FFPE) and for overnight at room temperature (FFPE). IHC assays against vimentin and cytokeratin were done without antigen retrieval. Western blot assays were done with whole extracts from same amounts of tissue samples fixed with respective method.

Under-fixation Does Not Count for Better Bio-molecules Preservation

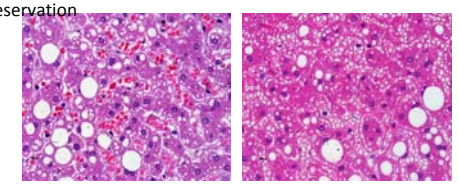

Figure 6. H&E staining of center and periphery of liver tissue fixed with and without US irradiation

Summary

- US-facilitated technology can greatly shorten the tissue fixation time from 6-48 hours to less than 30 minutes.
- Low temperature formalin fixation can keep patient specimens at low level of cross-linking status. Thus, it can overcome the drawback of excessive cross-linking of protein.
- The rapid tissue fixation can efficiently inhibit changes in macromolecular composition as well as tissue autolysis.

Acknowledgement

This research was supported by NIH/NCI CA091166-03 and CA091166-01