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Original contribution

Tissue tablet method: an efficient tissue banking procedure applicable to both molecular analysis and frozen tissue microarray☆,☆☆

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Summary Frozen human tissues are necessary for research purposes, but tissue banking methods have not changed for more than a decade. Many institutions use cryovial tubes or plastic molds with an optimal cutting temperature compound. However, these methods are associated with several problems, such as samples sticking to one another and the need for a larger storing space. We established an efficient tissue freezing and storing procedure ("tissue tablet method") applicable to both molecular analysis and frozen tissue microarray. Tissue samples were chopped into tiny fragments and embedded into tablet-shaped frozen optimal cutting temperature compound using our original tissue-freezing plate. These tablets can be sectioned and stored in cryovial tubes. We compared the tissue quality of tablet-shaped samples with that of conventional optimal cutting temperature blocks and found no significant difference between them. Tissue microarray is a key method to utilize tissue-banking specimens. However, most tissue microarrays require the coring out of cylindrically shaped tissues from formalin-fixed, paraffin-embedded tissue blocks. Antigenic changes and mRNA degradation are frequently observed with formalin-fixed, paraffin-embedded samples. Therefore, we have applied tablet-shaped samples to construct frozen tissue microarrays with our original mounting base. Constructed tissue microarray sections showed good morphology without obvious artifact and good immunohistochemistry and in situ hybridization results. These results suggest that the quality of arrayed samples was sufficiently appropriate for research purposes. In conclusion, the tissue tablet method and frozen tissue

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microarray procedure can save time, provides easy tissue handling and processing, and satisfies the demands of research methodologies and tissue banking.

1. Introduction

Development of biochemical technologies in genomics and proteomics is contributing to identification of disease-associated mechanisms at the molecular level. Well-preserved specimens of normal or neoplastic human tissues are absolutely necessary for implementation of these technologies. Many tissue banks have been established as national resources [1,2] and provide well-documented, high-quality human tissues to translate the study findings regarding cell lines or animal models into clinical applications. Although the technological, methodological, and logistic aspects of tissue bank applications have been improved, the procedures involved in the processing and storing of tissue specimens for research purposes have not changed materially for more than a decade [3-6]. Many facilities use cryovial tubes or plastic molds with a cryopreservation medium such as optimal cutting temperature (OCT) compound. The cryovial tube is easy to label and store in freezing boxes; however, it is difficult to remove specimens from the vial because they often stick to the vial wall or other specimens, possibly leading to an increased risk of thawing. Histological images can be assessed using the plastic molds, but the blocks require a large space for storage; therefore, it is difficult to store multiple samples of each specimen.

To solve the problems associated with these conventional methods, both cryostat sectioning to provide frozen sections and storage of multiple samples in minimal space are necessary to ensure the supply of an unused tissue for each sample ordered by researchers. In the present study, we used our original freezing plate with OCT compound to freeze tissue fragments into small tablets, then stored them in cryovial tubes. The objective of the present study was to demonstrate that our newly developed "tissue tablet method" (TT method) not only contributes to efficient tissue sample freezing and storing but also provides sufficient and similar sample quality as conventional OCT blocks.

Tissue microarray (TMA) is a key method to utilize tissue banking specimens. TMA is a useful technique that arrays a number of tissue cores in a recipient block and permits simultaneous comparison of the expression of target genes, proteins, or other investigation objects in a single experiment. Most existing TMA construction methods require coring out of cylindrically shaped tissues from formalin-fixed, paraffin-embedded (FFPE) donor blocks and transfer of the cores into a paraffin recipient block [7,8]. However, problems associated with antigenic changes and mRNA degradation may be induced by the fixation and embedding process [9] in FFPE TMAs. A few frozen TMA (fTMA) construction methods have recently been reported for better quality of tissue samples [10,11]. However, these methods require tissue cores that have been cut out from conventional OCT donor blocks; these block defects may not be permissible in every case because OCT blocks are very delicate and only one block is usually available for each specimen. In addition, re-sectioning of fTMA blocks is associated with the same problems as those of OCT blocks. Usually, an OCT block is directly fixed on a specimen holder of the cryostat by OCT compound and removed from the holder after sectioning for re-storing. However, the block is frequently damaged during its removal.

To solve these problems, both construction of an fTMA block without coring out of a cylindrical sample and maintenance of the block shape after sectioning for re-use are required. In the present study, we used TT method tablet tissues as donor samples and our original OCT block-mounting base to maintain the shape of the blocks. The second objective of this study was to demonstrate that our newly developed method contributes to easier fTMA construction without expensive devices.

2. Materials and methods

2.1. Tissue samples and freezing procedure

Twenty-one fresh tissue samples derived from 14 patients who underwent surgery between October and November 2011 at Kyushu University Hospital were collected for this study. There were 14 carcinoma tissues (6 pancreas, 2 esophagus, 5 colorectum, and 1 breast) and 7 normal tissues (1 pancreas, 2 esophagus, and 4 colorectum). Two different protocols were applied to the test, and the effects of different freezing methods were compared.

2.1.1. TT method

Several tissue pieces of each of the 21 samples were processed with our new method. We designed an original freezing plate (Meiko Medical, Fukuoka, Japan) (Fig. 1A) with 5 trapezoidal shallow pockets (top diameter, 13 mm; bottom diameter, 7 mm; depth, 3 mm) on a 50 × 50–mm square, fluorine-coated aluminum plate and used this plate for tissue freezing instead of plastic molds. To obtain adequately sized chopped tissues (~4 × 4 × 1 mm), we used a 4-mm graduated plastic sheet and double-edged razor (FA-10, Feather Safety Razor, Japan) (Fig. 1B). Chopped samples were placed into the shallow pockets of the freezing plate