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Technical report

Boric acid-enhanced embedding medium for cryomicrotomy

Jin Ik Lim, Hun-Kuk Park*

Department of Biomedical Engineering and Healthcare Industry Research Institute, College of Medicine, Kyung Hee University, Dongdaemun-gu, Seoul 130-701, Republic of Korea

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ABSTRACT

A polyvinyl alcohol (PVA)/polyethylene glycol (PEG)-based resin is commonly used as a cryoembedding medium for the histological analysis of frozen tissue sections. However, it is not easy to obtain sufficient numbers of satisfactory reproducible sections owing to the differences between the mechanical properties of the medium and embedded tissue and the low cohesive force of the medium. We describe a modified PVA-based cryoembedding medium, composed of PVA (10 wt% and 15 wt%) with the addition of boric acid (from 0 to 5 wt%), that can improve the sectioning properties and efficiency of frozen tissue for histological analysis. The amount of load under the same compressive displacement as well as cohesive force increased with increasing boric acid and PVA contents. 15 wt% PVA and 3 wt% boric acid was determined as an optimal composition for cryoembedding material based on the sectioning efficiency measured by the numbers of unimpaired sectioned slices and the amount of load under the same compressive displacement test. On the basis of the results of routine hematoxylin and eosin staining of cryosections of tissue embedded in a medium with 3 wt% boric acid and PVA, it was concluded that the modified PVA cryoembedding medium can improve the efficiency of cryosectioning for subsequent histological or histochemical analysis of various tissues.

Introduction

Three basic elements are generally required for successful histological analysis of tissues: a suitable embedding medium, a suitable sectioning method, and subsequent staining. Optical imaging, staining, and sectioning methods have been investigated for potential application in high-quality histology techniques, including immunohistochemical techniques, cryosectioning, and microscopy (Nicolas and Bassot, 1993; Sano, 1994; Yahiro and Nagato, 2005). However, there have been only limited reports on improving embedding media applicable to various types and sizes of tissue samples using simple and easy protocols.

Cryosectioning techniques are used extensively for producing unfixed sections of tissues and cells for subsequent histological, immunohistochemical, biochemical, and autoradiographic analyses (Sawady et al., 1988). These frozen sections are particularly useful for rapid pathological medical diagnosis. Although most tissues are easily sectioned in the frozen state, several cryosectioning problems have been noted by researchers. These include the tendency of sections to be easily broken or curling owing to the poor mechanical properties of commercial polyvinyl alcohol (PVA)/polyethylene glycol (PEG)-based embedding media. A further problem can occur in tissue or cells having high water content, with little or no (in the case of isolated or cultured cells) extracellular matrix proteins. Also, more improved resolution of the microscope for cryosectioned structural information is required through physicochemical change of medium. In order to overcome these problems, we made a study in which boric acid was added as an ionic cross-linker into PVA to enhance the properties of the embedding media.

This report describes a method that can improve sectioning efficiency using PVA/boric acid mixture as a cryoembedding medium. We undertook this study with the anticipation that problems such as breakage and curling of frozen sections would be reduced by the increased cohesive force of the embedding material after the addition of boric acid.

The objectives of this study were: (1) to determine the advantages and disadvantages of PVA/PEG-based commercial cryoembedding media; (2) to design a modified PVA based on the determination of the influence of the composition of PVA and boric acid on the sectioning efficiency; and (3) to evaluate the staining efficiency of the new media with sectioned natural tissue by carrying out a routine staining procedure. In our study, the mechanical properties of embedding media, sectioning efficiency, surface analysis, and staining test were determined. Our results show that a modified PVA medium containing boric acid can be used as an improved embedding medium for cryosectioning.

* Corresponding author.
E-mail address: sigmoidus@khu.ac.kr (H.-K. Park).

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Materials and methods

Chemical reagents

PVA was purchased from Junsei (Tokyo, Japan). Boric acid, polyethylene glycol (PEG), formaldehyde, hematoxylin, eosin, and ethanol (99 wt%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). These chemicals were used without further purification.

Preparation of PVA/boric acid and PVA/PEG media

PVA (MW: 1500) of 10 wt% and 15 wt%, and boric acid (0, 0.5, 1, 2, 3, 4, and 5 wt%) were mixed in an aqueous solution by fusion at 80 °C for 5 h. Each mixture was strongly stirred for 5 h at the same temperature to obtain a homogeneous phase. The homogeneous solution was poured in a mold (30 mm in diameter and 20 mm in height). The mixtures were then frozen at −20 °C for 30 min. As a control, PVA (MW: 1500) of 10.24 wt% and PEG-600 of 25 wt% were mixed and frozen by the same method with PVA/boric acid media.

Measurement of the amount of load under the same compressive displacement

Differences in the amount of load under the same compressive displacement were determined according to the PVA and boric acid contents. The test was performed using a texture analyzer (TA-HD, Stable Micro Systems, Godalming, Surrey, UK) with a 50-kg load cell, TA-51 needle rig, and software (Texture Expert, Stable Micro Systems). Frozen media blocks, 30 mm in diameter and 20 mm in height, were fabricated. The crosshead speed was 1 mm/min (down rate), and the amount of load at a depth of 1 mm was determined.

Measurement of sectioning efficiency by a simulated test

Mouse liver embedded either in PVA/boric acid or PVA/PEG media was continuously sectioned by a cryostat microtome (CM 3050-S, Leica, Bensheimk, Germany) to obtain 20 sections, each with a thickness of 5 μm. Sections of frozen liver with a surface area of approximately 10 mm × 15 mm were cut. Finally, the number of unimpaired sectioned slices was counted by the naked eye.

Atomic force microscopy (AFM) for surface analysis of a dried slice surface

We compared the surface roughness of PVA/boric acid sections and PVA/PEG sections, which were sectioned into 5-μm-thick samples by a cryostat microtome. After drying at room temperature for 2 h, an atomic force microscope (XE-100 AFM, PSIA, Suwon, Korea) was used to compare the roughness of the dried surfaces of the frozen sections. The non-contact mode was used, and 3D-image and roughness data analysis was carried out using an XEI image processing program (PSIA). Scanning conditions used were as follows: scan rate, 1 Hz; scan size, 20 μm × 20 μm, 2 μm × 2 μm; scan mode, C-AFM; data width and height, 256 pixels.

Staining efficiency of embedded tissue by PVA/boric acid and PVA/PEG media

Mouse livers were fixed in formalin, and then embedded in PVA/boric acid medium. Control blocks were fabricated using PVA/PEG medium as a control. Then, 5-μm-thick sections from each block were mounted on a glass microscope slide. After removing the embedding medium, sectioned liver was stained by routine hematoxylin and eosin staining method. The stained sections were observed using a light microscope (Microphoto-FXA, Nikon, Tokyo, Japan).

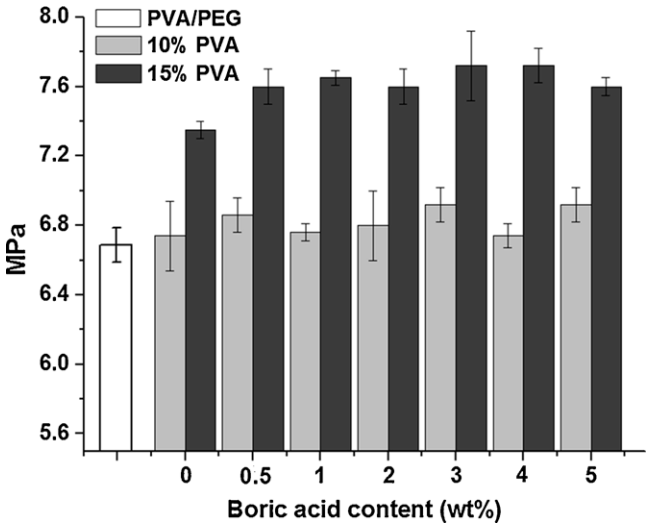

Fig. 1. Differences in the amount of load under the same compressive displacement according to boric acid and polyvinyl alcohol (PVA) contents.

Table 1
Number of unimpaired sections by a simulated test to test sectioning efficiency.

PVA/PEG	0%	0.5%	1%	2%	3%	4%	5%
10 ± 2	9 ± 2	10 ± 3	12 ± 2	12 ± 3	15 ± 2	14 ± 2	13 ± 1

Results

As cohesive force, under the same compressive displacement, the amount of load of the samples in 15% PVA/boric acid media was greater than that in 10% PVA media (Fig. 1). However, these frozen media showed that increasing the amount of load under the same compressive displacement occurred mainly by increased PVA content and was to a degree affected by the boric acid content. In addition, the value of the PVA/PEG medium as a control was shown to be similar to that of the 10% PVA/boric acid media.

The number of slices that remained unimpaired by continuous cutting increased after the addition of boric acid (PVA/PEG: 10 ± 2; 0 wt% PVA: 9 ± 2; 0.5%: 10 ± 3, 1%: 12 ± 2, 2%: 12 ± 3, 3%: 15 ± 2, 4%: 14 ± 2, and 5%: 13 ± 1, Table 1). The medium with 15% PVA/3 wt% boric acid showed the highest efficiency.

We then compared the surface roughness of PVA/PEG sectioned slices with sections of PVA/boric acid media using AFM. The arithmetical average roughness (Ra) of 15 wt% PVA/3 wt% boric acid sections was less than that of the PVA/PEG sections [Ra = 0.661 nm (PVA/PEG, 20 μm × 20 μm), Ra = 0.983 nm (PVA/PEG, 2 μm × 2 μm), Ra = 0.146 nm (PVA/boric acid, 20 μm × 20 μm), Ra = 0.251 nm (PVA/boric acid, 2 μm × 2 μm)]. Fig. 2a and b shows the 3D morphology of each sectioned slice surface at a 20-μm scale. Fig. 2a-1 and b-1 shows the magnified surface of the section at a 2-μm scale. Fig. 2a, a-1 shows the rough surface of the PVA/PEG sectioned slice. In contrast, the rough surface morphology clearly decreased by the addition of boric acid (Fig. 2b, b-1).

On the basis of the results of the staining tests, we concluded that it was not possible to differentiate the staining images of PVA/PEG-based commercial medium and PVA/boric acid medium-embedded slices based on the investigated staining method (Fig. 3).