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An improved approach to align and embed multiple brain samples in a gelatin-based matrix for simultaneous histological processing

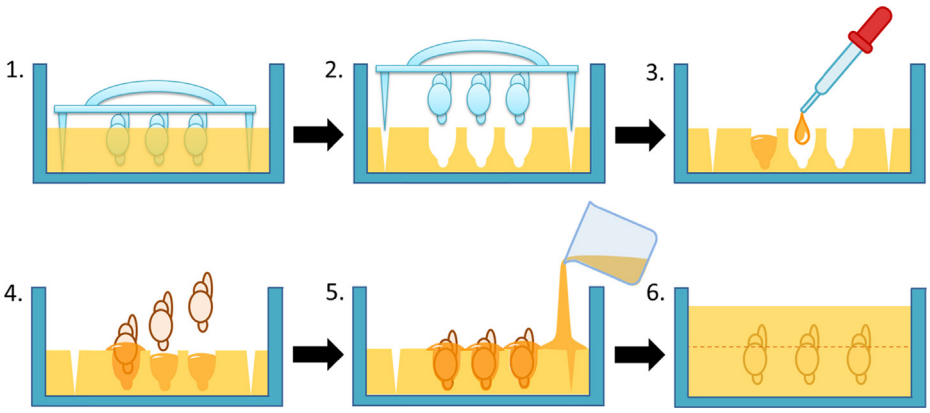
Kumi Nagamoto-Combs[a,*], Gunjan D. Manocha[b], Kendra Puig[b], Colin K. Combs[b]

[a] Department of Pathology, University of North Dakota School of Medicine and Health Sciences, 501N. Columbia Road Stop 9037, Grand Forks, ND 58202-9037, USA
[b] Department of Basic Sciences, University of North Dakota School of Medicine and Health Sciences, 501N. Columbia Road Stop 9037, Grand Forks, ND 58202-9037, USA

HIGHLIGHTS

- A method to simplify embedding of multiple brain samples was developed to facilitate histological processing.
- Templates were designed to form brain-shaped cavities in a gelatin-based embedding matrix.
- The cavities in the matrix allowed effortless positioning and alignment of brain samples during embedding.
- The gelatin matrix effectively secured the samples during sectioning, staining and mounting.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Preparation and processing of free-floating histological sections involve a series of steps. The amount of labor, particularly sectioning and mounting, quickly multiplies as the number of samples increases. Embedding tissue samples in a flexible matrix allows simultaneous handling of multiple samples and preserves the integrity of the tissue during histological processing. However, aligning multiple asymmetrical samples, for example small-animal brains, in a particular orientation requires skillful arrangement and securing of the samples by pinning onto a solid surface. Consequently, costly technical services offered by contract research organizations are often sought.
New method: An improved approach to align and embed multiple whole or half rodent brain samples into a gelatin-based matrix is described. Using a template specifically designed to form arrayed mouse brain-shaped cavities, a "receiving matrix" is prepared. Inserting brain samples directly into the cavities allows the samples to be effortlessly positioned into a uniform orientation and embedded in a block of matrix.

* Corresponding author. Tel.: +01 701 777 2559; fax: +01 701 777 6372.
E-mail address: kumi.combs@med.und.edu (K. Nagamoto-Combs).

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Results: Multiple mouse brains were arrayed in a uniform orientation in a gelatin matrix block with ease using the receiving matrix. The gelatin-embedded brains were simultaneously sectioned and stained, and effortlessly mounted onto glass slides.
Comparison with existing methods: The improved approach allowed multiple whole or half mouse brains to be easily arrayed without pinning the samples onto a solid surface and prevented damages or shifting of the samples during embedding.
Conclusions: The new approach to array multiple brain samples provides a simple way to prepare gelatin-embedded whole or half brain arrays of commercial quality.

1. Introduction

Histological analysis of biological tissue is essential for studying cellular morphology and phenotypes *in situ*. However, the process to prepare microscope-ready tissue samples is rather laborious, and more time and effort are required when a large number of tissue samples needs to be analyzed. In clinical pathology laboratories, tissue microarray, a method to "punch" out pieces of tissues and embed them in paraffin in an ordered arrangement, has been developed and is often utilized for simultaneous analysis of multiple biopsied specimens (Kononen et al., 1998; Miettinen, 2012). In basic biomedical research, where the analysis of whole organ tissues from experimental animals may be preferred, tissue embedding techniques have been more commonly used to protect the structure of complex organs such as the brain (Bjarkam et al., 2001; Griffioen et al., 1992) and cochlea (Hurley et al., 2003) during processing rather than to process multiple samples at the same time.

Recently, Smiley and Bleiwas (2012) have described a method to embed multiple mouse brains. In their protocol, the brain samples were secured on a solid surface with pins and embedded in a gelatin-based matrix containing several ingredients including albumin, lysine and glutaraldehyde. While they successfully demonstrated simultaneous sectioning and staining of multiple brain samples, the method requires each brain sample to have an extra tissue area (*e.g.*, brainstem) to be pinned or risk damaging a portion of the sample by the pinning process. This requirement poses a challenge when smaller samples such as neonatal or half-brain samples must be secured. Furthermore the inclusion of additional constituents to facilitate cross-linking of gelatin to the tissue samples makes the preparation of the matrix more complicated and costly.

In this report, we describe a new method to effortlessly align and embed multiple brain samples in a gelatin-based matrix block for simultaneous tissue processing. With two prototypes of specifically designed "brain array" casts, we first created a mold or "sample receiving matrix" with sample-shaped cavities in an organized arrangement. Using this technique, we were able to eliminate the pinning process and align small, mouse brain hemispheres without damaging any part of the tissue. The gelatin block with embedded samples can be ready for frozen sectioning within 3–4 days without many of the additional ingredients included in previously described embedding matrices (Smiley and Bleiwas, 2012).

2. Materials and methods

2.1. Materials

Gelatin (from porcine skin, type A, gel strength 300) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and used to make a gelatin-based embedding matrix. "Brain array casts" were specifically designed in order to align multiple mouse brains and embed them in a single block. Two prototypes tested in this study were constructed with high heat-sensitive polymer (high-temperature melting glue gun glue) and liquid rubber (Fig. 1). The rabbit polyclonal anti-mouse Iba-1 antibody was purchased from Wako Chemicals USA (Richmond, VA, USA) and used at a 1:1000 dilution. For immunohistochemistry, the anti-rabbit VECTASTAIN Elite ABC kit and ImmPact VIP were obtained from Vector

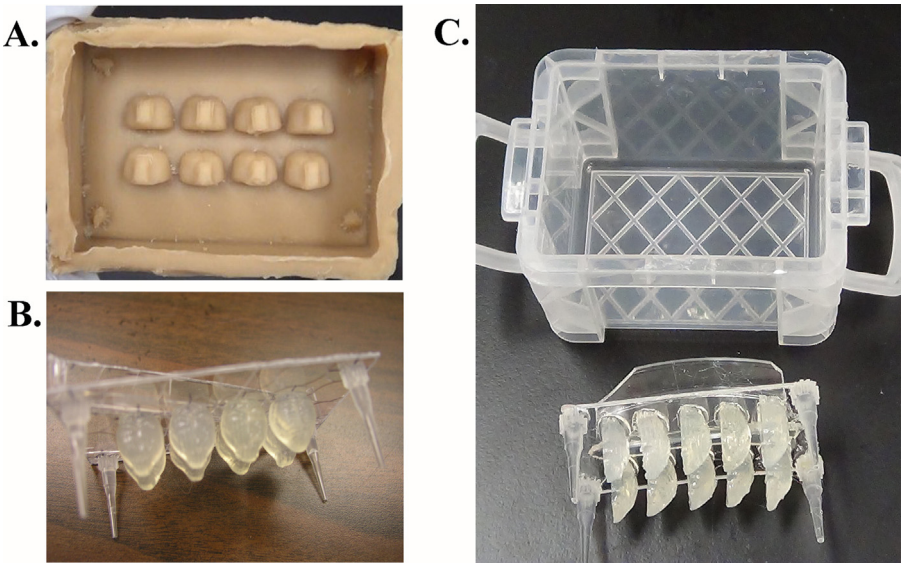

Fig. 1. *Brain array cast prototypes.* Devices for preparing sample receiving matrices were designed and prototypes were created from liquid rubber (A) and heat-sensitive polymer (B and C). The designs A and B create cavities that are the shape of whole brains, while design C forms half-brain shaped cavities in a sample receiving matrix. Design C, pictured with a small plastic container used as a receptacle, was used to demonstrate the embedding procedure.