Agar-gelatin for embedding tissues prior to paraffin processing

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Fixed tissues often need to be arranged in a particular configuration, order, or orientation prior to processing for paraffin embedding. Although it is possible to arrange tissues after infiltration (during the final stages of paraffin embedding), it can be more convenient and practical to do this at the time of dissection or tissue collection. Agar has been the classical medium used for this purpose (1–5). Typically, tissues are arranged or oriented as desired, and molten agar is poured over the top of the tissues. The agar solidifies around the fixed tissue (pre-embedding) and is then subjected to routine dehydration and paraffin infiltration procedures, followed by embedding the agar-encased tissue in paraffin wax. However, tissues that tend to shrink during processing (e.g., rodent spinal cord tissue) allow wax to infiltrate between the pre-embedding medium and the tissue (unpublished observation). Once sections are laid on a 42°C water bath prior to mounting onto microscopic slides, they rehydrate and expand more extensively than the agar and the layer of wax encasing them. As a result, some regions of the tissues do not lay flat on the slide and are subsequently either torn away or poorly stained during histological procedures (Figure 1A). Gelatin, on the other hand, is also not suitable, on its own, for pre-embedding, since its shape tends to warp, and it becomes very hard after processing for paraffin embedding (unpublished observations). We took advantage of the texture of agar and the low melting point of gelatin to make a pre-embedding matrix that would avoid the problems of each individual medium.

Bacto Agar (4%, w/v; BD Diagnostic Systems, Sparks, MD, USA) in distilled water at 40°C is mixed with a 5% (w/v) solution of gelatin A (300 Bloom; Electron Microscopy Sciences, Hatfield, PA, USA) in distilled water at a 1:1 ratio to generate a slightly viscous 2% agar:2.5% gelatin solution. The temperature of this medium is used at approximately 40°C to avoid exposing tissues to excessive heat. This medium can be stored at 4°C and remelted at least twice without altering its useful properties. Medium should not be used when it becomes very viscous or too cool, since the increased viscosity prevents the medium from entering small holes and spaces around the tissue, and avoiding these air pockets is crucial to both retaining tissue during processing and proper sectioning later. Perfused and 4% paraformaldehyde-fixed rodent central nervous system (CNS) tissues were used here as an example (using procedures approved by the Johns Hopkins University Animal Care and Use Committee).

Larger tissues (pieces of rodent brain that can stand on edge without toppling) are arranged in a mold (e.g., no. 27147-6; Ted Pella, Redding, CA, USA), and the pre-embedding medium is poured over the top of them. The thickness of the resulting block should be such that tissues are held firmly to allow their removal from the mold without tearing (4–6 mm). Once firmly solidified (≥10 min on a cold surface), the medium around the tissue is trimmed with a razor blade. Agar-gelatin tissue blocks are stored at 4°C submerged in 150 mM NaCl in cassettes until ready for dehydration.

For smaller tissues or tissues that might move from their prearranged position after having embedding medium poured over them, a variation of this technique can be helpful (Figure 2). Rodent spinal cord segments, for example, can be pre-embedded to maintain their rostral-caudal order and to keep them standing on end (for cutting in cross-section) during paraffin processing. Agar-gelatin blocks are cast in molds (Figure 2, step 1). Cores of solidified medium are made with a Miltex biopsy punch (Miltex, York, PA, USA), Harris Uni-Core™ (Electron Microscopy Sciences), or other cutting tool at 3 mm diameter for mouse spinal cord and are removed with pointed forceps (Figure 2, step 2). Tissue pieces are placed in the wells that are created, leaning on the well’s walls (Figure 2, step 3). Pre-embedding medium is slowly added along the well wall opposite from the tissue to fill in

Figure 1. Comparison of pre-embedding with agar alone versus agar-gelatin mixture. (A) Agar alone results in folds and poor adherence of sections of mouse thoracic spinal cord (arrows), while (B) agar-gelatin pre-embedded sections lay flat on the slide. Sections are stained with hematoxylin and eosinY.

Figure 2. Method for pre-embedding rodent spinal cord segments in agar-gelatin pre-embedding medium. (1) Agar-gelatin medium is poured over rodent spinal cord segment. (2) Medium is trimmed with razor blade to create a core around the tissue to maintain its rostral-caudal order during processing. (3) Tissue is placed in well of mold, leaning on wall and gently filling in remaining space with medium. (4) Tissue is removed from mold for dehydrating and paraffin infiltration.
Once sections are cut and rehydrated as for blocks containing only paraffin, dehydration is performed in the same manner by the preceding alcohol-mediated and embedding because it is fixed it survives paraffin infiltration (60°C). Gelatin in an aqueous solution melts at 40°C, the agar portion of the embedding media may persist. However, these will not interfere with analysis of the tissue, because the tissue itself is not infiltrated with the pre-embedding medium.

Overall, the use of a pre-embedding medium empowers the investigator to retain information regarding the orientation and location of the tissues that would otherwise be lost after tissue collection. Agar alone may be suitable for pre-embedding tissues in some cases, but tissues that shrink during processing require the added flexibility provided by a mixture of agar and gelatin to allow the re-expansion of the tissue and proper mounting onto slides. Ultimately, pre-embedding procedures allow the investigator to control how tissue, especially multiple pieces of tissue, are arranged and oriented in the final paraffin block.

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES


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