Quick and Easy Adaptation of Molecular Biology Technology to Tissue Embedment Polymerization

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Abstract.

Using the SPI Cure™ with a standard heating block for 1.5 snap-cap conical tubes, at temperatures from 55–59°C, we have achieved safe and complete polymerization of LR White (acrylic) and SPI Pon (epoxy) resins in both protected and unprotected BEEM® capsules as well as 00-gelatin capsules. Please note that this report concerns a novel[3] adaptation of dry-block heating technology designed for molecular biological procedures. In this test, we have purposefully used only resin blanks. Finally, we report that in the case of LR White, no degradation of the material of which the BEEM® capsules are constructed has been observed in either 'protected' or 'unprotected' heating regimes. We recommend careful testing of embedding molds not specifically mentioned in this paper.

Introduction.

One of the problems with the preparation of small batches/numbers of plastic-/resin-embedded specimens for Electron or Light Microscopy is that the same polymerizing equipment—rotators, processors, and ovens (vacuum and standard) must be used. Also, with large or small batches, the exclusion of oxygen is a more or less common problem in all successful polymerization protocols. We describe a simple, and inexpensive means by which the thermally accelerated polymerization procedure can be accomplished using a standard dry-block heater. The same close tolerance required for temperature control (0.1°C) in many molecular biological procedures can now be utilized by EM technicians who choose to follow our suggestion. If vacuum infiltration is required, it may be accomplished prior to use of the SPI Cure™. Dry-block heaters come in many flavors and capacities. We have chosen to introduce one that is the smallest size and perfect for up to 12 specimens. Further, these heaters are sufficiently compact that even large ones can easily be moved into a relatively small space in a chemical fume hood of almost any size.

Methods and Materials.

For this experiment, we have used standard processing equipment and procedures except for the means by which we apply heat to polymerize the resins. All polymerizations were performed at temperatures between 55°C and 59°C (we avoided 60°C!) in a compact dry-block heater equipped with two independently regulated heaters, one in the base and one in the cover (see photo attached). Gelatin capsules and the smallest BEEM® capsule were filled with LR White or SPI Pon, closed, and then enclosed in standard 1.5ml conical snap-cap tubes. For all other BEEM® capsules, we used cut-off parts of the same snap-cap-tubes to keep the caps of the BEEM® capsules above the surface of the aluminum heater block. We have tested two resins: LR White (the "Hard" Formulation of the acrylic resin) and, SPI Pon (an epoxy replacement for EPON 812), both available from SPI Supplies. In both of these tests, successful polymerization occurred 'overnight' (approximately 16 hours) with no loss of resin or visible deformation of either BEEM® or gelatin capsules or snap-cap tubes. Capped enclosures were used to prevent chemical damage to the plastic cover of the dry-block heater.

Results and Conclusions.

We have used a standard off-the-shelf dry-block heater (now named the SPI Cure™) to successfully polymerize two commonly used plastic embedments. The SPI Cure has been shown to be a safe tool to be used to completely polymerize LR White and SPI Pon resins in 00-gelatin capsules and all of the standard BEEM® capsules. This, we believe, is a novel application of a standard dry-block heater used as a substitute for the large ovens traditionally used for the polymerization step in sample processing. Further, since the time required for polymerization is no different from that achieved with larger ovens, the use of this technology provides a 'greener' energy signature for this part of specimen preparation. Without any attempt to provide a statistical comparison, we note that a ~1 ft^3 vacuum oven in our lab draws 12A while the small block heater draws only 2A. It is anticipated that the system will work for other embedding media that require heat-assisted polymerization.

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3. We have only searched for similar use of dry-block heaters using "dry-block heater polymerization" in Google.