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A Multisample Chamber for Dehydration and Critical Point Drying

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ABSTRACT The principles and methods for constructing an improved chamber for dehydration and critical point drying of multiple biological samples are described. The specimen chamber design is based on vertical positioning of the electron microscope grids or coverslips and permits minimal perturbation of laminar solvent flow past the specimens. This condition is requisite for optimal exposure of samples to solvents, which is necessary for complete dehydration and drying. Fragile samples, including chromosomes, critical point dried in the multisample chamber demonstrate crisp, well-preserved, three-dimensional morphology.

INTRODUCTION

Critical point drying is a technique used to preserve the three-dimensional structure of specimens to be examined in the electron microscope (Anderson, 1951). Commercially available sample holders are usually expensive, designed for processing small numbers of samples and inadequately constructed to facilitate solvent flow. We have designed a relatively inexpensive chamber which easily holds up to 100 electron microscope (EM) grids. Our model fits in most commercially available critical point dryers and holds EM grids, but it could easily be modified for use in other critical point dryers and to handle other substrates, such as coverslips. Our specimen chamber design permits minimal perturbation of laminar solvent flow past the specimens. This characteristic is essential for good critical point drying and has not been optimized in commercially marketed chambers.[1]

MATERIALS AND METHODS

The following materials were used during construction of the multisample chamber: two 5.8-cm segments of large diameter round brass tubing (pipe; outer diameter (OD) = 2.54 cm; inner diameter (ID) = 2.03 cm); twenty-seven 6.35-cm lengths of thin wall, square brass tubing (OD = 0.3175 × 0.3175 cm); flat head machine screws, slotted, stained steel (#2–56 × 1.27 or 2.54 cm); hex nuts, stainless steel (#2-56); flat washers, stainless steel (size 2W; Small Parts, Inc., Miami, FL); bronze insect wire screening (14 × 18 mesh; Alamo Iron Works, Houston, TX); jigging putty (Bowman Distribution Co., Cleveland, OH); solder (281 steel bond; X-Ergon, Irving, TX); ballpoint pen springs (0.6-cm segments); and #600 carborundum paper.

Assemble the base of the gridholder by placing the square brass tubes in one segment of brass pipe and stabilize this assembly by pushing it into a patty of jigging putty (see finished assembly, Fig. 1A). Heat the brass pipe with an acetylene torch so that solder flows smoothly and fills the gaps between the tubes and the pipe (but does not enter the brass tubes). Proper heating allows the solder to fill the small spaces between the tubes by capillary action. When the assembly is cool, wash the putty off under running water. Cut off the base and then cut at

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[1]Ladd Research Industries, Inc. (Post Office Box 1005, Burlington, VT 05402) is introducing a commercially available multisample chamber for dehydration and critical point drying based on the principles of the unit described in this paper.

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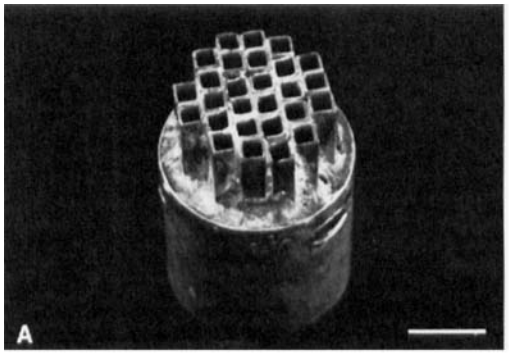

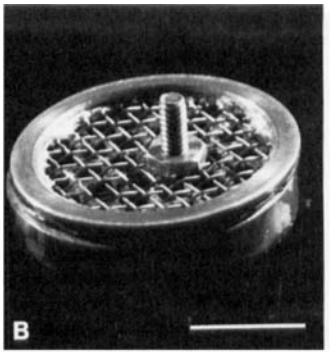

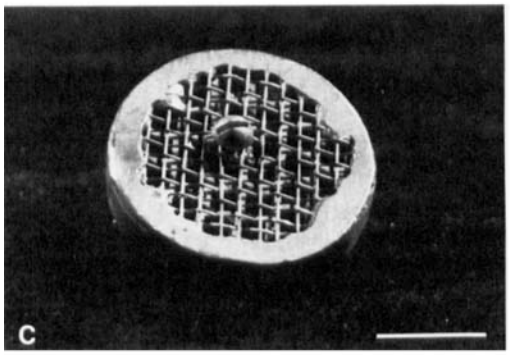

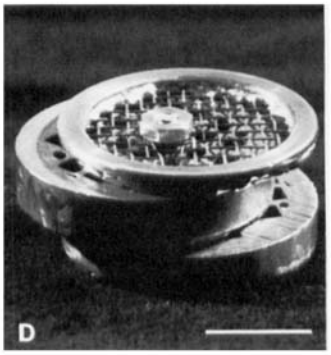

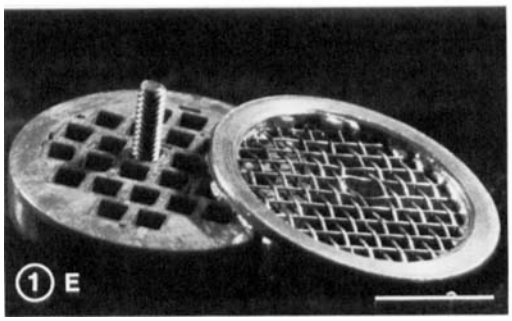

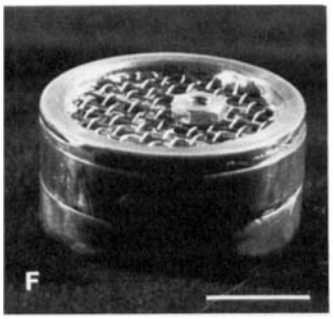

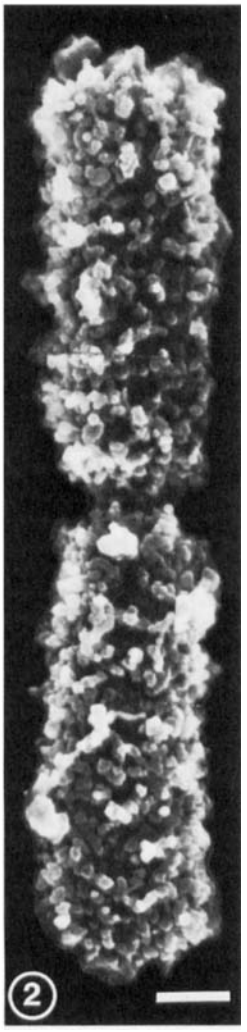

Fig. 1. Photograph of (A) soldered assembly of 27 square brass tubes in brass pipe from which gridholder sections were cut; (B) assembled single-section unit (optional spring/washer assembly around screw is absent); (C) bottom view of gridholder, note off-center positioning of screw and soldered attachment of screen to section of square tube-pipe assembly; (D) assembled double section unit, skewed to show grid chambers and that the screen from the upper gridholder section forms a cover for the lower section; (E) single gridholder unit, note square chambers in which grids were placed from corner to corner and top of unit, composed of brass ring to which brass screen was soldered; and (F) fully assembled two-section unit that holds 52 grids. Bars, 1 cm.

Fig. 2. Scanning electron micrograph of a whole-mounted metaphase chromosome isolated from cultured Chinese hamster Don cells by nitrogen cavitation using the pH 6.8 method of Wray and Stubblefield (1970). Specimens were centrifuged onto a formvar-coated grid, stabilized in 2% uranyl acetate, dehydrated in a graded series of acetone solutions, critical point dried in the multisample chamber by the method of Anderson (1951) using liquid CO_2 as the intermediate fluid, and sputter-coated with 12–15 nm of gold-palladium. Specimens were examined using a JEOL JEM-100CX scanning–transmission electron microscope and the images were recorded on Polaroid Type 55 positive/negative film from a 1000-line cathode ray tube. Bar, 5 μm.

least five 4-mm sections from the assembly using a lathe.

Prepare the tops of the gridholders by cutting at least five 1-mm sections from the unused segment of brass pipe. Reem out the holes of the gridholder bases using American–Swiss pattern files (Heller Tool Div.) and polish the brass rings and the bases using carborundum paper. Cut 2.54-cm circles from the copper screen using scissors. One screen is then soldered onto the perimeter of each gridholder base and top, so that the gridholder and screen holes remain solder-free (see Figs. 1C and 1E). Use carborundum paper to sand the solder flush with the screen and sides of the brass pipe. Enlarge a hole in the screen beneath one of the central brass tubes using forceps and place a 1.27-cm screw through the screen and the base of the gridholder (Figs. 1C and 1E). Enlarge a similar hole in the screen on the gridholder top and assemble the chamber (Fig. 2B; note that both screens face down). Place a hex nut, a washer, and a spring (optional) on the screw and then tighten the nut to hold the gridholder top in place. A larger capacity chamber may be constructed by using a 2.54-cm or longer screw and stacking two to four gridholder bases and a top on the screw before installing and tightening the hex nut-spring closure (Figs. 2D and 2F). A notch should be cut in the brass pipe, and/or rows of brass tubes should be numbered for convenient orientation of the gridholder. Grids are placed from corner to corner in each square brass tube.

To adapt our gridholder to fit other critical point dryers, simply choose brass pipe with an outer diameter that allows at least 0.159-cm clearance from each of the dryer walls and pack the maximum number of square tubes into the pipe. The most efficient method of modifying the gridholder to accommodate coverslips is to use rectangular brass tubes (eg, 4.76 × 9.53 mm) for 10.5 × 22 mm coverslips rather than square ones and cut the appropriate length (eg, 24 mm) sections of the pipe/tube assembly rather than 4 mm ones.

RESULTS AND DISCUSSION

Cells and organelles critical point dried using the multisample chamber exhibit crisp ultrastructural features. The metaphase chromosome in Figure 2 has well-preserved three-dimensional structure and coiled chromatin fibers.

The multisample chamber described in this paper has several advantages over commercially available gridholders. First, commercial gridholders are constructed in such a way that the grids sit flat in cylinders drilled vertically through a cross-sectioned metal rod. Solvent flows vertically through the cylinders, around the horizontally-positioned grids, creating turbulent solvent flow and inadequate exposure of the specimens to dehydrating and critical point drying fluids. In addition, rapid draining of solvents may flatten samples. In our sample chamber, grids are positioned vertically (in the direction of flow) and therefore produce minimal turbulance. These conditions are necessary for optimal exposure of samples to solvents and, thus, complete dehydration and drying, which are critical for good preservation of three-dimensional tissue ultrastructure. Second, the horizontal positioning of grids in commercial chambers makes handling of conventional EM grids difficult, and grids with tabs are required. Conventional grids are easily manipulated in our chamber because of the vertical grid position. Third, our sample chamber is relatively inexpensive, each tier costing approximately one-seventh as much as commercial gridholders. Finally, our chamber can hold as many as 104 EM grids; whereas the largest capacity commercial sampleholder that we could find has two tiers and holds only 24 EM grids, while costing approximately ten times as much as our single-tier model that holds 26 grids.

In conclusion, we have discussed the design and construction of a relatively inexpensive specimen chamber that holds a large number of electron microscope grids and permits adequate solvent flow past the samples. The ultrastructure of specimens prepared in the multisample chamber appears to be well-preserved.

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