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SILICONIZING GLASSWARE TO BE USED FOR SUSPENSION CELL CULTURE

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SUMMARY: Siliconizing glassware to be used for suspension cell culture with the reagent dichlorodimethylsilane after rigorous cleaning of the glass results in a more homogenously treated glass which will survive many detergent washings. The method described was found to be superior to other ways of coating culture glassware.

Key words: suspension culture; dichlorodimethylsilane.

I. INTRODUCTION

Cells are frequently cultured in suspension for experiments in cell biology, pharmacology, and biochemistry because this method of culture yields large quantities of cells with a minimum investment in equipment. Furthermore, cells grown in suspension are easily harvested and manipulated for use in transport and binding experiments (1-6). A common difficulty encountered with suspension cultures is the binding or attachment of the cells or cellular debris to the walls of the culture vessel. This problem may be minimized and in many cases avoided entirely by siliconizing the culture vessels.

A variety of commercial and specialty reagents have been utilized for treating glassware (1). For this treatment to be consistently effective we have found that the treatment must be preceded by properly preparing the surface of the glass. The method described has been found to be effective with Pyrex, Kimax, and soft (lime) glass. Once properly treated with the siliconizing agent, dichlorodimethylsilane, the glassware retains the silicon treatment for up to 10 detergent washings in hot water. The glassware so treated has been found to be nontoxic to a number of eukaryotic cell lines: HeLa (S3), L5178Y mouse lymphoblasts, mouse neuroblastoma, and Krebs 2 Ascites cells.

II. MATERIALS

Aluminum foil
Beaker, graduated, Griffin low form, 1000 ml No. 575516, Ann Arbor

III. PROCEDURE

A. Preparation of solutions

1. Alcoholic potassium hydroxide cleaning solution (7):

   a. In a glass beaker, gradually dissolve 56 g of potassium hydroxide in 70 ml of distilled water with constant stirring. If possible, this should be done in a cold room or in an ice bath. In any event, keep cool. CAUTION: Wear protective glasses and clothing!

   b. Dilute with stirring to 1000 ml with 95% ethyl alcohol.

   c. Store in an amber screw-capped reagent bottle. Stable at room temperature for at least 14 days.
2. Dichlorodimethylsilane:
   a. Dilute to approximately 1% concentration with toluene.
   b. Store in an amber bottle with a tightly fitting cap containing a polyethylene or teflon liner. CAUTION: This compound is volatile and corrosive; store and work with it only in an approved chemical hood!

B. Glassware preparation
1. Glassware is washed with an acceptable detergent to remove loose material, scrubbed if necessary, and rinsed thoroughly with tap and distilled water, and air dried.
2. Fill or immerse glassware completely with alcoholic potassium hydroxide. Place small objects in beaker or glass trough. CAUTION: Wear protective clothing and glasses!
3. After at least 30 min, the alcoholic potassium hydroxide may be returned to the stock container for reuse. The solution may turn brown without losing its efficacy but any precipitate should be removed (by decanting off the clear solution).
4. Glassware is thoroughly rinsed with tap and distilled water and dried completely in an oven. Clean, dry glassware is stored in a closed cabinet to avoid a build up of dust. Flasks and culture vessels are covered with aluminum foil.

C. Siliconizing glassware
1. To clean, dry culture vessels or other glassware to be coated, add 3 to 5 ml of 1% dichlorodimethylsilane dissolved in toluene. Carefully swirl to allow the reagent to contact all of the surface to be treated. CAUTION: Wear protective clothing and glasses and work in a hood!
2. Return reagent to stock container for reuse.
3. Rinse vessels or glassware with a small volume of toluene. Discard rinse in organic solvent waste disposal.
4. Allow vessel or object to thoroughly air dry (upside down in the hood, at least 30 min). Drying and removal of toluene fumes may be expedited by subjecting vessels to a stream of filtered air or inert gas.
5. Sterilize glassware using conventional methods. Vessels may be sterilized with dry heat at 180° C for 3 h without adverse effects.

IV. DISCUSSION
The treatment of glass with dichlorodimethylsilane replaces hydroxyl groups of hydrated silica with dimethyl silica anhydrides (8). This makes the surface less hydrophilic resulting in less binding of cells and cellular debris to the glass.

The method described for siliconizing glassware has been successfully used in our laboratories for 14 years with a variety of culture vessels such as Florence flasks and spinner bottles (1,2,5). We have found that this method is superior to the use of aqueous siliconizing agents [such as Siliclad6, Dricote6, or silicon stopcock grease (6)], because coverage is more uniform, excessive deposits are avoided, inadvertent transfer of silicon agents to other glassware is minimized and the treatment is more long lasting. Glassware may be washed many times with a nonalkaline detergent, sterilized, and reused without the necessity of resiliconizing. Furthermore, there are apparently no biochemical or morphological effects on the cells cultured in treated culture glassware.

The only disadvantages to this method are the cautions noted in the procedure, and its unsuitability for coating plastic or rubber culture equipment. In our experience, the latter is rarely important.

The silicon can be removed, if necessary, by cleaning the glassware with alcoholic potassium hydroxide (1).

V. REFERENCES
6. Morse, P. A.; Potter, V. R. Pyrimidine metabolism in tissue culture cells derived from rat hepatomas. I. Suspension