Replica Techniques for TEM

Introduction

When it is not practical or possible to put certain specimens into the TEM, the replica technique may be employed to examine surface features. The technique is useful for both biological and nonbiological materials but each material may require special preparative treatment.

Replicas may be negative (single stage) or positive (double stage) and a number of materials may be used to make these replicas. The materials and methods employed in this exercise are most suitable for non-biological materials or for hard, dry biological materials. The principles employed, however, are pertinent to other biological replica techniques.

Materials needed

Cellulose acetate replicating tape (3-4% Collodion or Duco cement may be substituted but take longer to dry)
Polished metal (stainless steel, aluminum or copper)
0.5 N HNO_3 or HC1
Pipettes
Small glass dishes
Acetone
Grids
Platinum-carbon evaporant (chromium or germanium may be substituted)

Procedures

A. Control

A piece of polished, cleaned metal is provided as the substrate to be replicated.

1. Wet a strip of replicating tape with acetone and apply it with light pressure to the metal.

2. After it dries completely (10-15 min.), strip it from the substrate, turn the replica over and tape it to a glass slide as shown in the following diagram.

   Note: It is wise to discard the first replica as bits of dirt may be embedded in the cellulose acetate.

B. Etched Surface

1. When all students have replicated the smooth metal, place several droplets of 0.5 N HC1 or HNO_3 onto the surface. Wash the acid off with clean water after ca. 15-30 seconds. Blow dry or place in oven to dry.

2. Replicate this surface as in part A and prepare the replica for evaporation by taping it to a glass slide. Make sure each replica is labelled.

C. Evaporation onto Replicas

1. Place both replicas into the vacuum evaporator at a distance from the metal electrode ca. 3 times the height of the evaporant. A carbon-fitted electrode should be directly above the replicas at a height of ca. 10-15 cm.

2. Pump down the evaporator and coat the specimen with a medium carbon layer followed by a shadow of platinum-carbon (Note: chromium or germanium may be substituted but will not take as "fine" a coat).

3. Remove the replicas and digest the cellulose acetate negative with acetone. There are a number of ways to do this, we suggest the first method below although the second is also reliable.

   a. Cut 2-3 mm squares from the replica with razor blades. Carefully set these on copper grids (300-400 mesh) and place these on acetone wetted filter paper in a glass petri dish. Keep the paper wet with acetone and periodically move the grid to a new position on the paper. In ca. 1 hr or less the acetate is all dissolved and the replica is ready for examination in the TEM.

   b. Cut large segments (1 cm square) from the replica and float them on a dish of acetone. The replica should readily separate and float on the top. A transfer pipette may be used to move the floating replica fragments after ca. 5 minutes. Place them first in a dish of clear acetone for 1-2 min., then drop them onto a clean water surface. They will flatten out and portions of the replica can be picked up from below with a forceps-held copper grid (300-400 mesh).

ASSIGNMENTS

Examine the replicas in the TEM. Take comparable fields of etched and unetched material surfaces. Include low and high mag. micrographs in the portfolio. Use an arrow to indicate the direction of shadowing.

REFERENCES

1. Ladd, M. W. 1973. The electron microscope handbook. pp. 18-30. Ladd Research Industries.

SHADOW-CASTING

The technique of shadow-casting consists of depositing by vacuum evaporation a layer of electron-dense material on to the specimen at an angle. It can be seen from the diagram that areas shielded from the impinging beam of atoms by surface irregularities are not coated. These areas are more electron-transparent than the coated areas and resemble shadows in appearance. When examined in the electron microscope, they will appear lighter than the surrounding areas. When a micrograph is taken of such a specimen, the images of the shadows are reversed and appear dark, thus giving the impression of a surface illuminated obliquely by white light.