Microsurgery on Protozoa Using Cactus Spines

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Gary Schoenwolf has published a method for preparation of cactus-spine microsurgical needles for use in surgical manipulation of chick and frog embryos (Schoenwolf and Watterson, 1989, *Laboratory Studies of Chick, Pig, and Frog Embryos*, Macmillan). Here we describe an application of cactus-spine needles in the teaching laboratory for study of cell reorganization and regeneration of the giant protozoan, *Spirostomum*, following microsurgical bisection.

Select a cactus plant that has a variety of spine sizes. Use a fine forceps to remove a number of spines of various sizes for mounting. You will learn by experience what types and sizes of spines work best for you.

Ordinary wooden applicator sticks (e.g., Fisher Scientific, catalog #01-340) make good handles for the spines. Split and slightly sharpen the end of the stick with a single-edge razor blade. Use clear nail polish to glue a spine in place in the split end of each stick. (We use Revlon Super Nails Natural Wonder.) An angle of approximately 45° of spine to stick is a good one for starters. Cactus-spine needles can be sterilized by dipping them in alcohol and letting them air dry, but not flame sterilized!

Spirostomum can be observed and manipulated in spring water in a depression slide. Note especially the prominent contractile vacuole which is clearly visible near the posterior end of the cell. The presence of a contractile vacuole in each of the two cells within 2 or 3 hours following bisection will serve as an easily observable sign of cell reorganization and regeneration.

A *Spirostomum* cell can be cut in half by laying a cactus-spine needle across the cell and pressing down. Sometimes a moving cell can be cut by one quick stroke, but often the organism contracts and avoids being cut. A more reliable technique involves gently touching the organism and then quickly cutting across it during the momentary pause after it has stopped moving and contracted strongly in response to the initial touch.

The two cut cell halves can be left in the depression slide if it is placed in a covered petri plate lined with a folded wet paper towel to retard evaporation. Repeated examinations over the next several hours will allow observation of cell reorganization and regeneration.

We have done only a little testing of cactus-spine needles in other microsurgical procedures, but generally we think that cactus-spine microsurgical needles substitute quite well for drawn glass needles or needles made of sharpened tungsten wire. Some cactus-spine needles seem a bit too flexible for certain operations, but because of the ease with which they can be constructed, we foresee a bright future in the teaching laboratory for “Nature's microsurgical needles.”