SCLEROBLAST CULTURES FROM THE GORGONIAN LEPTOGORGIA VIRGULATA (LAMARCK) (COELENTERATA: GORGONACEA)

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SUMMARY

Scleroblasts were separated from fragmented tissue of growing tips of Leptogorgia virgulata and cultured using a modification of the technique of Rannou. Replacement of fetal bovine serum with horse serum seemed to increase scleroblast viability. Cell adhesion occurred from 14 to 43 d. Cultured scleroblasts demonstrated cell aggregation, spicule formation, and extrusion of spicules into the external medium. Cells showing spicules in the process of being extruded appeared on the average after 24 d of culture. Variability among cultures was marked with respect to both division and spicule formation. Healthy cultures were maintained for more than 4 mo.

Key words: cell culture; gorgonian; Leptogorgia virgulata; scleroblast.

INTRODUCTION

The octocoral Leptogorgia virgulata, a gorgonian or sea whip, forms elaborate polycrystalline, calcitic spicules (7). Initial formation and growth of these microscopic structures occur individually within a vacuole of a scleroblast, the spicule-forming cell (7). Mature spicules, however, have been shown to make the transition from the intracellular to the extracellular environment (7). Rannou (11) described spicule formation in cultured cells from fragments of planula larvae of the gorgonian Eunicella and found the spicules to be similar to those of young polyps. However, he did not discuss spicule formation in scleroblasts derived from the mesoglea of adult branches.

Leptogorgia virgulata has proven to be an extremely useful organism for diversified investigations of the mechanisms of calcification in invertebrate systems [see (6) for review]. Establishment of a culture method for the mesogleal scleroblasts from actively calcifying apical portions of the branches of these animals would provide large quantities of easily accessible material for experimental studies of mineralization. This paper describes the culture of mesogleal scleroblast cells from L. virgulata using a modification of the method of Rannou (11).

MATERIALS AND METHODS

Collection and maintenance. Colonies of L. virgulata were collected manually at low tide from Sixty Bass Creek of North Inlet estuary, Georgetown County, South Carolina, and from subtidal waters off Morehead City, North Carolina. They were transported in aerated seawater to the laboratory where they were placed in large aquaria of filtered seawater and kept at 17°C. Crushed oyster shells were used around the bases of the colonies to maintain an upright position. Within 60 min of transplanting the animals into aquaria, polyps emerged on all colonies.

The laboratory diet consisted of three parts decomposing Spartina (marsh grass) to one part mud from marshland adjacent to the collection site. This material was processed in a blender and then boiled for 5 min. One to 100 ml of the mixture/20 gal seawater was added to the aquaria daily. Alternatively, animals were maintained on a commercially prepared liquid diet formulated for filter feeding organisms (Marine Invertebrate Diet; Hawaiian Marine Imports, Inc., Houston, TX).

Culture methods. Branch tips approximately 3 cm in length were cut from actively feeding aquaria-adapted animals, rinsed in membrane-filtered (0.45 μm) seawater, and placed in a solution of sterile seawater containing antibiotics for 1.5 to 2 h. The solution consisted of 2000 U of penicillin G (Sigma), St. Louis, MO; 5 mg of dihydrostreptomycin sulfate (Sigma); 25000 U of Colimycin (Sigma); 500 U of Bacitracine (Sigma); and seawater to make 100 ml. The pH was adjusted to 7.5 with sodium bicarbonate. This solution was sterilized by passage through a Nalgene filter unit (0.45 μm). The branches were then transferred to a sterile petri dish, covered with this antibiotic solution, and the “tissue” was removed from the axial skeleton with a sterile razor blade. The tissue was then macerated by chopping with a razor blade into fragments of approximately 0.05 mm³. The fragments were passed through a 16-gauge needle using a minimal amount of sterile medium. Preformed extracel-
lar spicules which averaged 110 \( \mu \text{m} \) in length readily separated from the tissue upon maceration and most were removed by hand centrifugation. The uppermost tissue portion of the pellet was then placed in culture dishes, covered with medium, and examined for spicules. The composition of the medium contained identical concentrations of the antibiotic solution described above plus 20 ml T. C. 199 (GIBCO, Grand Island, NY), 10 \( \times \) concentrate, diluted to 5 \( \times \) with sterile seawater and 11 ml of horse serum. In earlier cultures, 11 ml of fetal bovine serum was used in the medium instead of horse serum. Cultures were maintained in 30-ml (Falcon) or 25-cm\(^2\) (Corning) tissue culture flasks. To facilitate subsequent scanning electron microscopy (SEM) observations, 50 mm diameter Belco culture dishes, with circular mylar sheets fitted to the bottom, were employed. For the 1st wk, 5.0 ml medium was replaced daily with 5.0 ml fresh medium. Three times during the 2nd wk the medium was similarly replaced. Thereafter, until cell adhesion occurred, the cultures were fed three times a week with new medium devoid of antibiotics. Once the cells adhered to the culture flask, the medium was completely poured off, along with any remaining free spicules, and fresh medium was added. Cultures were maintained at 21 °C and exposed to 12 h of light and 12 h of darkness.

Cell viability was determined by trypan blue exclusion and, in those cultures not killed, by appearance and behavior (phase brightness, division, aggregation, spicule formation).

At intervals, an average of 500 cells were counted and the percentage of those viable, dividing, and forming spicules was recorded. Only those with a clear, visible possession of spicules were counted as spicule-forming cells in this study. In preparation for SEM, cells adhering to the mylar sheets were subjected to critical-point dehydration using ethanol and \( \text{CO}_2 \) with a LADD critical point dryer, and coated with gold and examined with JEOL JSM-U3 and Hitachi S-570 scanning electron microscopes.

RESULTS

An average of 15.5% of the scleroblasts cultured in medium containing fetal bovine serum was viable after cell adhesion compared to an average of 36% viability for those in medium containing horse serum. Fetal bovine serum was therefore replaced with horse serum and the following results describe cultures after this replacement was made. Cell adhesion occurred from 14 to 43 d. Cell adhesion within the 1st wk was not frequent but occurred 10% of the time. Viable cell cultures have been maintained for over 4 mo. A typical mature scleroblast measured 40 to 80 \( \mu \text{m} \) in diameter (Fig. 1). Scleroblasts were often seen in aggregations (Figs. 1 and 2).

Results from 10 representative cultures from three of numerous experiments (group A, B, and C) are shown in Fig. 3. The percentage of cells undergoing division (Figs. 1, 2, 4) varied from culture to culture. This variability existed within and between the culture groups (Fig. 3). In group A, the number of cells undergoing division immediately after adhesion, in general, increased with time as seen in Fig. 3 b–d. However, in culture A 1 (Fig.

Fig. 1. Scanning electron microscopy of aggregating and dividing scleroblasts. \( \times \)420.

Fig. 2. Light micrograph of scleroblast culture showing cells dividing, aggregating, or associated with extracellular spicules or both (arrows). \( \times \)100.