GROWTH RATE, LABELING INDEX, AND RADIATION SURVIVAL OF CELLS GROWN IN THE MATRIGEL THREAD IN VITRO TUMOR MODEL


Department of Radiation Oncology (J. J. C., L. K. C., J. C. L., D. B., J. S. R.) and Radiology (R. G. S.), University of Washington Medical Center, Seattle, Washington 98195; Laboratory of Molecular Pharmacology, Building 37 Room 5C25, National Cancer Institute, Bethesda, Maryland 20892 (J. J. C.); and Department of Diagnostic Imaging, St. Jude Children's Research Hospital, 332 N. Lauderdale, P.O. Box 318, Memphis, Tennessee 38101 (R. G. S.)

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SUMMARY

Six rodent cell lines (36B10 rat glioma cells, 9L rat gliosarcoma cells, V79 Chinese hamster lung fibroblasts, EMT6/UW and EMT6/Ro mouse mammary sarcoma cells, and RIF-1 mouse fibrosarcoma cells) were tested for growth in cylindrical threads of Matrigel. These cells grew in the threads with doubling times of 17-23 h, reaching maximum cell densities on the order of 10^8 cells/ml. Histological sections of these threads showed a heterogeneous cell distribution: cells grew to confluence at the thread surface and at somewhat lower cell densities in the thread core. [H-3]thymidine labeling index and radiation sensitivity were measured for 9L and EMT6/UW cells in Matrigel threads. For both cell types, the labeling index in Matrigel was lower than observed in cell monolayers, with higher labeling indexes at the thread periphery than in the thread core. When these threads were grown in stirred medium, lower thread diameters, higher cell yields per thread, and higher labeling indices were obtained. EMT6 cell monolayers coated with Matrigel were less radiosensitive than cells in uncoated monolayers. This protective effect was eliminated by irradiating in the presence of 1 mg/ml misonidazole. EMT6 cells consume nearly three times as much oxygen (mole/cm^2-sec) as do 9L cells, which are equally radiosensitive in monolayers with or without a Matrigel coating. The radiation sensitivity of EMT6/UW cells in Matrigel threads was similar to that for monolayers of plateau phase cells, whereas for 9L cells, the response in threads was more similar to exponentially growing cells. We conclude that Matrigel threads provide an alternative in vitro model for studying the radiation response of cells in a three-dimensional geometry.

Key words: Matrigel, tumor, radiation response, EMT6, 9L.

INTRODUCTION

Matrigel is a solubilized basement membrane preparation, extracted from the Engebreth-Holm-Swarm mouse sarcoma, containing laminin, collagen IV, heparin sulfate proteoglycans, enactin, and a variety of growth factors (Kleinman et al., 1982; McGuire and Seeds, 1989). Matrigel has been shown to cause differentiation in human endothelial cells (Grant et al., 1989) and carcinoma cells (Hohn et al., 1992; Kibbey et al., 1992). Several human tumor cell types have been shown to grow in Matrigel (Daly et al., 1988; Chou et al., 1989; Parodi et al., 1989; Vijayakumar et al., 1992; Topley et al., 1993; Uladag and Setfon, 1993). Metastatic tumor cells are also able to invade the gel (Reich et al., 1988; Parodi et al., 1989; Melchiori et al., 1990; Noel et al., 1991). Daly et al. (1988) and Vijayakumar et al. (1992) have demonstrated that human tumor cells can be grown in cylindrical Matrigel threads at high enough cell densities to acquire 31P-NMR spectra.

Magnetic resonance (MR) spectroscopy provides a potentially useful tool for investigating tumor response to chemotherapy (Wehrle et al., 1987; Steen, 1989) and tumor oxidation-reduction state (Livesey et al., 1989). Several in vitro tumor models have been used for MR spectroscopy, including cells on microcarrier beads (Ugurbil et al., 1981), agarose encapsulated cells (Foxall et al., 1984; Lyon et al., 1986), multicellular spheroids (Freyer et al., 1990), hollow fiber bioreactors (Gillies, 1993), and Matrigel threads (Daly et al., 1988; Vijayakumar et al., 1992). These models vary in their resemblance to in vivo growth conditions and in their degree of microenvironment heterogeneity. An ideal model would have cell densities sufficient for MR studies (on the order of 10^6 cells/ml), a healthy and relatively homogeneous cell population, and cells that can be harvested for clonogenic assays. In this work, the growth of several rodent tumor cell lines in Matrigel threads was characterized in order to determine the utility of this model for irradiation survival and MR spectroscopy experiments.

MATERIALS AND METHODS

Cell types used. Six cell types were used in this study: EMT6/Ro (Dr. Robert Sutherland, University of Rochester Cancer Center) and EMT6/UW mouse mammary sarcoma cells (Dr. Robert Kallman, Stanford University), 9L rat gliosarcoma cells (Dr. Henry Brem, Johns Hopkins University), 36B10 rat glioma cells (Dr. Alexander Spence, University of Washington), V79-171B al., 1987; Steen, 1989) and tumor oxidation-reduction state (Livesey et al., 1989). Several in vitro tumor models have been used for MR spectroscopy, including cells on microcarrier beads (Ugurbil et al., 1981), agarose encapsulated cells (Foxall et al., 1984; Lyon et al., 1986), multicellular spheroids (Freyer et al., 1990), hollow fiber bioreactors (Gillies, 1993), and Matrigel threads (Daly et al., 1988; Vijayakumar et al., 1992). These models vary in their resemblance to in vivo growth conditions and in their degree of microenvironment heterogeneity. An ideal model would have cell densities sufficient for MR studies (on the order of 10^6 cells/ml), a healthy and relatively homogeneous cell population, and cells that can be harvested for clonogenic assays. In this work, the growth of several rodent tumor cell lines in Matrigel threads was characterized in order to determine the utility of this model for irradiation survival and MR spectroscopy experiments.

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Chinese hamster lung fibroblasts (Peggy Olive, British Columbia Cancer Control Agency, Vancouver, BC) and RIF-1 mouse fibrosarcoma cells (Dr. J. Martin Brown, Stanford University). Eagle's minimum essential medium (EMEM) with 10% fetal bovine serum (FBS) was used for 9L, V79, and RIF-1; Waymouth's medium with 15% FBS was used for EMT6/UW and 36B10; and basal medium Eagle (BME) with 15% FBS was used for EMT6/Ro. FBS was obtained from Flow Laboratories (McLean, VA) and all medium was obtained from Sigma Chemical Co. (St. Louis, MO) or Gibco (Grand Island, NY).

**Matrigel thread preparation and growth.** Standard Matrigel (Collaborative Research, Bedford, MA), as opposed to growth-factor-depleted Matrigel, was used in all experiments. Matrigel is a liquid at 4°C, but gels rapidly and irreversibly at room temperature. Thus, Matrigel was stored frozen and thawed at 4°C prior to thread preparation. Precleaned pipettes and test tubes were used for handling Matrigel, and Matrigel was kept on ice during thread preparation. The technique used for seeding cells in Matrigel threads was similar to that used by Daly et al. (1988). A 200 μl aliquot of a cell suspension containing 0.1 ml Matrigel/ml was added to 1 ml of Matrigel at 4°C. The mixture was then drawn into a 10-15 cm length of 20-gauge Teflon tubing and allowed to harden in the tubing at room temperature for 3 to 4 min. The thread was then slowly extruded into a plastic petri dish (Falcon, Becton Dickinson, Oxnard, CA, not treated for cell attachment) containing 5 ml growth medium at 37°C. Threads were then incubated at 37°C for up to 8 d. In some cases, the medium was removed from the petri dish and replaced with 5 ml of fresh medium daily starting at the 3rd day of growth. In one experiment, the medium was stirred continuously during thread growth by incubating the threads on an orbital shaker.

**Thread volume measurement.** The thread diameter was measured at 10 randomly selected positions along the thread length using a calibrated reticle and the mean diameter was computed. Because the threads were not straight, their length was determined using digitization and image analysis. Each thread was stained with 0.05% trypan blue for 3 to 5 min to improve contrast during imaging. The thread and a ruler were then photographed using a Javelin model JE2062 TV Camera with a Fujon TV Zoom lens and an image was obtained for a Macintosh II using either Computer Eyes (Digital Vision, Dedham, MA) or MacVision (Koala Technologies, Morgan City, CA) software. Image (Version 1.29, Dr. Wayne Rasband, NIH Research Services Branch, Bethesda, MD) was used to determine the thread length from the digitized image.

**Cell harvesting and counting.** Three solutions were tested for dissociating threads into single cell suspensions: Dispase (50 units/ml), a bacillus-derived neutral protease obtained from Collaborative Research; 10 mg/ml neutral buffered Type IX protease (Sigma, 1 unit/mg) in medium; and 1.5 g/liter trypsin with 0.6 g/liter EDTA (3X trypsin/EDTA, Sigma) in Ca2+Mg2+-free Hank's balanced salt solution (HBSS). Each thread was cut into small sections and added to a 125 ml Erlenmeyer flask containing a 1-inch magnetic stir bar and 30 ml disaggregation solution. The solution was stirred at 1200 rpm and 37°C for either 45 min (Dispase and protease) or 30 min (trypsin) to dissociate the threads. Cells were centrifuged (3 min at 800 rpm), resuspended, and counted using either a hemocytometer or a Coulter counter.

**Cell viability assay.** One ml of a 1:20 dilution of Matrigel in growth medium was added to 35-mm tissue culture dishes and the solution was incubated at room temperature for 1 h. After aspirating 0.5 ml of the unbound solution, the dishes with the remaining 0.5 ml of Matrigel were incubated at 37°C overnight. Roughly 8 × 105 cells were added along with 1.5 ml growth medium to dishes with and without Matrigel and allowed to grow for 3 d. The cells were then irradiated at a dose rate of 1.5 Gy/min at doses of 0 to 24 Gy using a 60Co irradiator equipped with a rectangular 20 × 7.5 cm collimator (Shephard and Associates, Glendale, CA, Model 61-14). In some experiments, misonidazole (1 mg/ml) was added 15 min prior to irradiation. After irradiation, the cells were plated for colony formation. The surviving fraction was calculated as the ratio of the average plating efficiency at any given dose to that for the unirradiated activity = 20 Ci/mMol, New England Nuclear, Boston, MA) for 30 min at 37°C with agitation of medium. This was followed by a 1-h incubation in label-free medium. Threads were then fixed in neutral buffered formalin, embedded, sectioned at 5 μm, dipped in Kodak NTB2 emulsion, and exposed for either 4 h (for grain density measurements) or 7 d (for labeling index measurements). Slides were developed in Kodak D-19 developer and stained through the emulsion with hematoxylin. To insure that a section through the thread center was examined, the diameter of each thread section was measured and only thread sections with diameters greater than 90% of the known value, allowing for a 25% shrinkage due to histology, were used. The labeling index (LI) was determined by dividing cell location in the thread into two regions: peripheral cells, defined as those cells at a distance no greater than five cell layers from the thread surface and core cells being the remainder. Clumps of cells projecting out from the thread surface and clusters of cells near the broken ends of the threads were excluded from the analysis. The LI of exponential and plateau phase cells in monolayer (2 × 105 cells seeded 3 or 6 d prior to the experiment in a 25 cm² tissue culture flask containing 5 ml medium) was measured by exposing cells to 1 μCi/ml [3H]-thymidine for 30 min. This [3H]-thymidine concentration was sufficient to discriminate between labeled and nonlabeled cells. In all cases, [3H]-thymidine was at tracer amounts.

**Radiation survival measurements.** The radiation response of 9L and EMT6/UW (B,D) cells grown as Matrigel threads with either daily growth medium replenishment (closed symbols) or no growth medium replenishment (open symbols).

![FIG. 1. Thread diameter (A,B) and cell number per thread (C,D) for 9L (A,C) and EMT6/UW (B,D) cells grown as Matrigel threads with either daily growth medium replenishment (closed symbols) or no growth medium replenishment (open symbols).](image-url)