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THREE-DIMENSIONAL GROWTH PATTERNS OF VARIOUS HUMAN TUMOR CELL LINES IN SIMULATED MICROGRAVITY OF A NASA BIOREACTOR

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SUMMARY

Growth patterns of a number of human tumor cell lines that form three-dimensional structures of various architectures when cultured without carrier beads in a NASA rotary cell culture system are described and illustrated. The culture system, which was designed to mimic microgravity, maintained cells in suspension under very low-shear stress throughout culture. Spheroid (particulate) production occurred within a few hours after culture was started, and spheroids increased in size by cell division and fusion of small spheroids, usually stabilizing at a spheroid diameter of about 0.5 mm. Architecture of spheroids varied with cell type. Cellular interactions that occurred in spheroids resulted in conformation and shape changes of cells, and some cell lines produced complex, epithelial-like architectures. Expression of the cell adhesion molecules, CD44 and E cadherin, was upregulated in the three-dimensional constructs. Coculture of fibroblast spheroids with PC3 prostate cancer cells induced tenascin expression by the fibroblasts underlying the adherent prostate epithelial cells. Invasion of the fibroblast spheroids by the malignant epithelium was also demonstrated.

Key words: spheroid architecture; suspension culture; low-shear culture.

INTRODUCTION

Three-dimensional growth of cells or tissue *in vitro* is now an established culture method that has proved advantageous in many research applications (1,4,16,22). In the case of tumor cells, such three-dimensional cultures, often referred to as spheroids, have been found to be more like small tumors *in vivo* than are cells grown in conventional monolayer cultures. Methods for culturing cells as three-dimensional structures include static culture on or in a gel matrix with liquid overlay or in agitated suspension as in roller bottles or stirred cultures (1,24,25). Recently, as part of the National Aeronautics and Space Administration's (NASA) Microgravity Science and Applications program, a new low-shear, suspension culture method and a family of bioreactors were developed. Designed to mimic microgravity, these rotary cell culture systems are proving to have wide applicability in research concerned with cellular interactions in tissue-like constructs (3,9,18,20). Our laboratory at the Huntington Medical Research Institutes was privileged to have early access to this technology, allowing us to gain several years' experience with its use in culturing a variety of cell types and, increasingly, with cocultures. We describe here the growth patterns of a number of human tumor and stromal cell lines that form three-dimensional structures of various architectures when cultured without carrier beads in the NASA rotary cell culture system.

MATERIALS AND METHODS

The bioreactor. The NASA bioreactor used for studies reported here is referred to as the HARV (high aspect ratio vessel) (Fig. 1). The reusable culture chamber is a very shallow cylinder, approximately 9 cm in diameter, between rigid Lexan walls. One of the walls is solid Lexan; the other is a ring with its central portion replaced by a silicone gas exchange membrane. The two walls are screwed firmly together, sealed by an o-ring. During culture, 5% CO_2 in air is pumped across the membrane to ensure adequate oxygen supply and gas exchange. The culture chamber is fitted with three ports, two of them accessed via luer syringe fittings through which media can be added or removed. The third port has a somewhat larger orifice and can be used when spheroids are harvested. The culture chamber volume is 50 ml. In operation it is completely filled, i.e., bubble-free. The culture chamber screws firmly onto a motor-driven rotator which rotates the chamber slowly about a horizontal axis throughout the culture period. In addition to the rotator motor, the base of the system holds a pump which directs the CO_2/air mixture in the tissue culture incubator over the surface of the silicone oxygenation membrane. The culture chamber is sturdy and autoclavable and the silicone membrane can be replaced when necessary. The NASA-developed bioreactors are now available commercially from Synthecon, Inc., Houston, Texas.

Culture procedure. To start a culture, the investigator completely fills the chamber by syringe through one of the ports, making sure that all bubbles are eliminated. The optimal cell concentration is between 5×10^5 and 2×10^6 cells per milliliter of medium appropriate for the cell line. The filled culture chamber is screwed onto the rotator and the system is placed in the tissue culture incubator at 37° C. Rotation is initially approximately 15 revolutions per minute. As spheroids form and enlarge during culture, the rate of rotation is increased as necessary to keep the particles suspended and essentially in free fall. Usually the rotation rate can be maintained at or below 25 rpm. The entire contents of the culture chamber rotates with the vessel so that shear forces within the vessel are very small. Medium is removed and fresh medium added by syringe approximately once daily via the luer syringe fittings. Cultures are maintained for periods of a few days to approximately a month.

Cell lines cultured and cocultured. Sixteen cell lines from either the Huntington Medical Research Institutes or the American Type Culture Collection were cultured in one of two media (Table 1). One experiment reported here is a coculture in which a monodisperse suspension of the prostate cancer cell line, PC3, was added to spheroids of normal prostate fibroblasts that had formed during the preceding 4 d of culture in the bioreactor. In this experiment, the bioreactor was seeded with 30×10^6 NPF fibroblasts and the

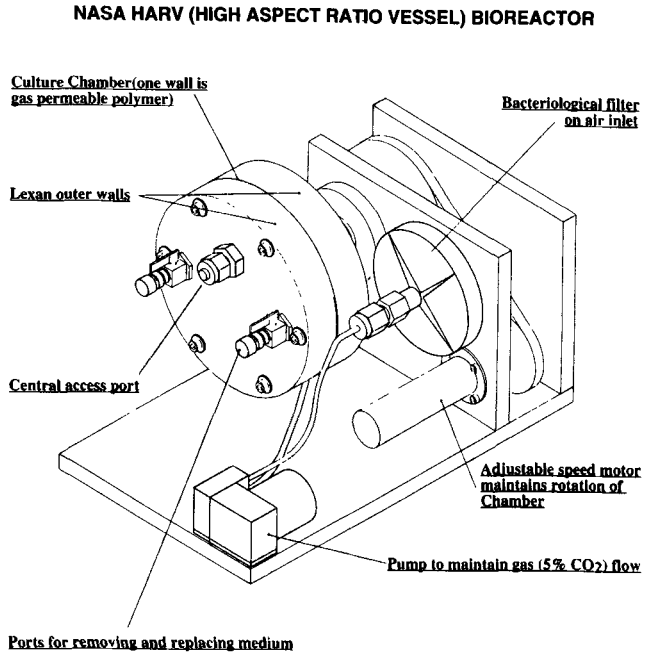

FIG. 1. The NASA HARV (high aspect ratio vessel) bioreactor used in culturing a variety of cell lines.

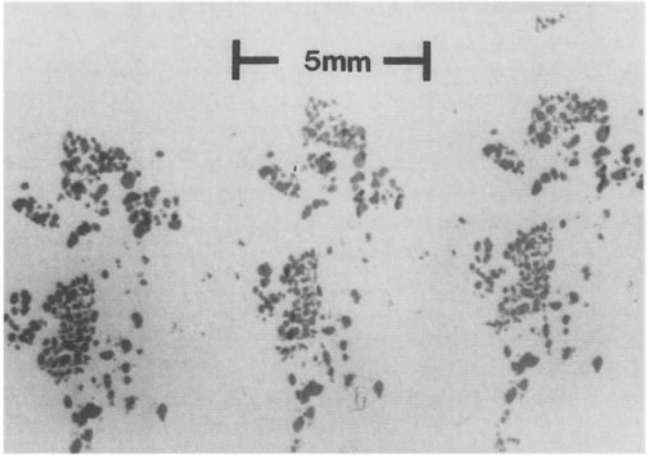

FIG. 2. An entire harvest of spheroids of the breast cancer line BT20 was processed as a batch and sectioned. The spheroid diameters of these H & E stained sections range from approximately 0.1 mm to 0.4 mm. The large field showing the three sections was made with a lensless microscope (KENSAL Corp., Tucson, AZ, courtesy of Kendall Preston, Jr., President). This electronic imaging system is uniquely capable of imaging the area of an entire rectangular coverslip.

culture maintained for 4 d. At that time, 39×10^6 PC3 cells in suspension were added to the 4-d fibroblast spheroids and the culture was continued for another 4 d. This experiment was the first in an ongoing series of cocultures concerned with interactions between cancer cells and stromal cells. In all other experiments reported here, cultures were maintained without microcarriers of any sort to avoid anchorage effects.

Fixation and staining. Harvested spheroids were fixed in buffered formalin or Bouin's fixative and sectioned for staining. Representative sections were routinely stained with hematoxylin and eosin. Gene products of interest were demonstrated immunohistochemically in duplicate sections with appropriate polyclonal or monoclonal antibodies. Specific antibody dilutions ranged from 1:50–100 to 1:200–400, and antigen retrieval pretreatment was used for two of the eight antigens tested (Table 2). Microwave pretreatment, when used, was for 10 min in 100 m*M* sodium citrate buffer (pH 6.0) in a home-model microwave oven with subsequent cooling (for 20 min) to room temperature. Bound antibodies were demonstrated with protocols contained in the Vector Elite™ (peroxidase) kit (Vector Laboratories, Burlingame, CA) and stained with 3′,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO).

Markers were selected for immunostaining on the basis of their specificity for certain cell types or as differentiation signatures. Interpretation of immunohistochemical staining was based on conventional microscopy to estimate the percentage of cells that stain with a given antibody and the intensity of the staining. Results were expressed with a scale of one plus to four plus. To evaluate further the differential effect of three-dimensional growth on cell morphology and on expression of various markers, centrifuged pellets of freshly harvested monolayer-grown cells as well as monolayer-grown cells grown and fixed in culture chambers (Lab-Tek Permanox® slides, NUNC, Inc., Napeville, IL) were also stained immunohistochemically and compared with spheroids grown in the rotating cell culture system.

Scanning electron microscopy. Scanning electron microscopy (SEM) was done as described previously (12). Monolayers of cells were grown on 13-mm glass coverslips to near confluency before being fixed for SEM. Spheroids harvested for SEM were allowed to adhere to polylysine-coated glass coverslips (1–2 h at 37° C) before fixation. Initial fixation was with 2% glutaraldehyde, 1% paraformaldehyde, and 1 m*M* $CaCl_2$ in 0.1 *M* sodium cacodylate buffer, pH 7.4. Following a brief buffer rinse, the samples were postfixed with 1% osmium tetroxide in 0.1 *M* sodium cacodylate buffer, pH 7.4. The coverslips of cells were next dehydrated in a graded series of ethanol; dried (critical-point method or directly from hexamethyldisilazane), and sputter-coated with gold-palladium before examination in the SEM.

RESULTS

Spheroid size and structure. Small particles appeared within a few hours after a culture was started. During the first week they enlarged by cell division and by fusion of small spheroids to form larger aggregates. Thereafter, spheroid size tended to stabilize within a size range that varied among cell lines. When cells formed spheroidal structures, the maximum diameter of the individual spheroids was approximately 0.5 mm, but was usually about 0.3–0.4 mm. In some cases the smaller spheroids fused to form larger bodies that were sometimes elongated or irregular. Some cell lines formed structures that were more like sheets of tissue and these tended to be larger than true spheroids. Fig. 2 shows the typical size range of cancer cell spheroids formed by the breast cancer cell line, BT20. These cells were cultured in a smaller, 10-ml bioreactor for 7 d. The figure shows that substantial harvest of spheroids is attainable with the low-shear suspension culture system. Some spheroids fragmented, especially in cultures maintained for several weeks. Not all cell lines produced particles that were truly spheroidal. Some, like the prostate cancer cell line, LNCaP, were more like folded sheets of cells (Fig. 3 *a*) and some, such as the prostate cancer cell line, PC3, formed extremely loose aggregates that behaved as discrete particles although cells seemed scarcely in contact with each other (Fig. 3 *b*). In contrast, DU 145 prostate cancer cells formed compact, round spheroids (Fig. 3 *c*). Compact spheroids tended to have an outer zone one or a few cells thick, in which cells were more elongated and formed a pseudocapsule. This was a prominent characteristic of spheroids formed by gliomas and fibroblasts and by some epithelial cells; it was less prominent in spheroids of cells such as LNCaP that formed folded sheets. It was completely absent in PC3 spheroids.

Mitotic figures occurred more frequently among the cells in the peripheral zone, and mitotic cells in the outermost layer were some-

TABLE 1

HUMAN CELL LINES CULTURED IN HARV BIOREACTOR

Cell Line	Age (yr)	Sex/Race	Source	Passage[a]	Medium and Additives[b]
Malignant gliomas					
HBR 9	62	m/c[c]	HMRI[d]	8–11	DMEM-F12 + 10% FBS
HBR 84	56	m/c	HMRI	7–14	DMEM-F12 + 10% FBS
HBR 65	62	m/c	HMRI	8–24	DMEM-F12 + 10% FBS
HBR 51	51	m/c	HMRI	9	DMEM-F12 + 10% FBS
HBR 147	43	m/c	HMRI	9–24	DMEM-F12 + 10% FBS
01 T	59	m/c	HMRI	7–10	DMEM-F12 + 10% FBS
02 T	65	m/c	HMRI	5–20	DMEM-F12 + 10% FBS
Prostate cancer					
PC3	62	m/c	HMRI	14	DMEM-F12 + 10% FBS
LNCaP			ATCC[e]#1740	24	DMEM-F12 + 10% FBS
DU 145			ATCC#HTB81	68	DMEM-F12 + 10% FBS
Urinary bladder cancer					
HBL 2	74	m/c	HMRI	6–14	DMEM-F12 + 10% FBS + EGF 4 ng/ml
J 82			ATCC#HTB-1	58	DMEM-F12 + 10% FBS + EGF 4 ng/ml
Metastatic brain tumor					
HBM 10	60	f/c	HMRI	5–12	DMEM-F12 + 10% FBS
Breast cancer					
BT 20			ATCC#HTB19	High	DMEM-F12 + 10% FBS
Normal prostate fibroblasts					
NPF 209	20	m/c	HMRI	4	DMEM-F12 + 10% FBS
Coculture					
PC3/NPF209			HMRI	15/4	DMEM-F12 + 10% FBS

[a]Multiple cultures of most cell lines.

[b]DMEM-F12 medium was purchased from GIBCO, Gaithersburg, MD, and FBS (fetal bovine serum) was purchased from HyClone Laboratories, Inc., Logan, UT. Human epidermal growth factor (recombinant) was purchased from Upstate Biotechnology, Inc., Lake Placid, NY.

[c]Caucasian.

[d]Huntington Medical Research Institutes (HMRI) cell lines:

- HBR series of cell lines as well as 01T and 02T originated from surgical specimens obtained during a phase I and phase II clinical trial of immunotherapy for recurrent malignant glioma (11). All tumors were glioblastoma multiforme.
- The prostate cancer cell line, PC3, was obtained at autopsy from a metastatic lesion (grade IV adenocarcinoma) in bone of a 62-year-old white male (12). This cell line is now available from ATCC (1435). At the time the cell line was established, HMRI was known as the Pasadena Foundation for Medical Research.
- NPF 209 cells were obtained at autopsy from the normal prostate gland of a 20-year-old Caucasian male victim of a traffic accident.
- HBL2 originated from a surgical specimen (radical cystectomy); tumor was an invasive, poorly differentiated transitional cell carcinoma.
- HBM10 originated from surgical specimen of a metastatic brain tumor in an adult Caucasian woman; primary site unknown but probably epithelial (tumor cells were negative for glial fibrillary acidic protein and vimentin; 2+ for cytokeratin and 2–3+ for carcino-embryonic antigen).

[e]American Type Culture Collection.

TABLE 2

ANTIBODIES USED FOR IMMUNOHISTOCHEMISTRY

Antigen and Dilution	Clonality	Supplier[a]	Description and Specificity
Cytokeratin VIII [35 βH 11] (1:50–100)	Mono.	Dako	54-kDa protein; nonsquamous epithelial tumors
Cytokeratin High mol mass [34 βE12] (1:100)	Mono.	Dako	Keratins of approx. 66 kDa and 57 kDa; squamous, ductal, and other complex epithelial neoplasms
GFAP (1:200–400)	Mono.	Dako	52-kDa intermediate filament protein; glial fibrillary acidic protein (GFAP) in brain and spinal cord
Tenascin (1:50–100)	Mono.	Dako	1900 kDa; mesenchymal cell lines
Collagen IV (1:50–100)	Mono.	Dako	Collagen IV; characteristic staining of basement membranes in a variety of tissues
E Cadherin[b] (1:200)	Mono.	Zymed	Human epithelial Cadherin; (E cadherin or uvomorulin)
CD44[b] (1:100)	Mono.	Novo	Human Cell Adhesion Molecule (HCAM) human T cells, leukocytes, and some tumor cells
PCNA (1:100)	Mono.	Dako	Polymerase delta accessory protein (36 kDa); present in all proliferating cells

[a]Dako, Corpinteria, CA; Zymed, South San Francisco, CA; Novo, Newcastle upon Tyne, UK

[b]Microwave antigen retrieval.

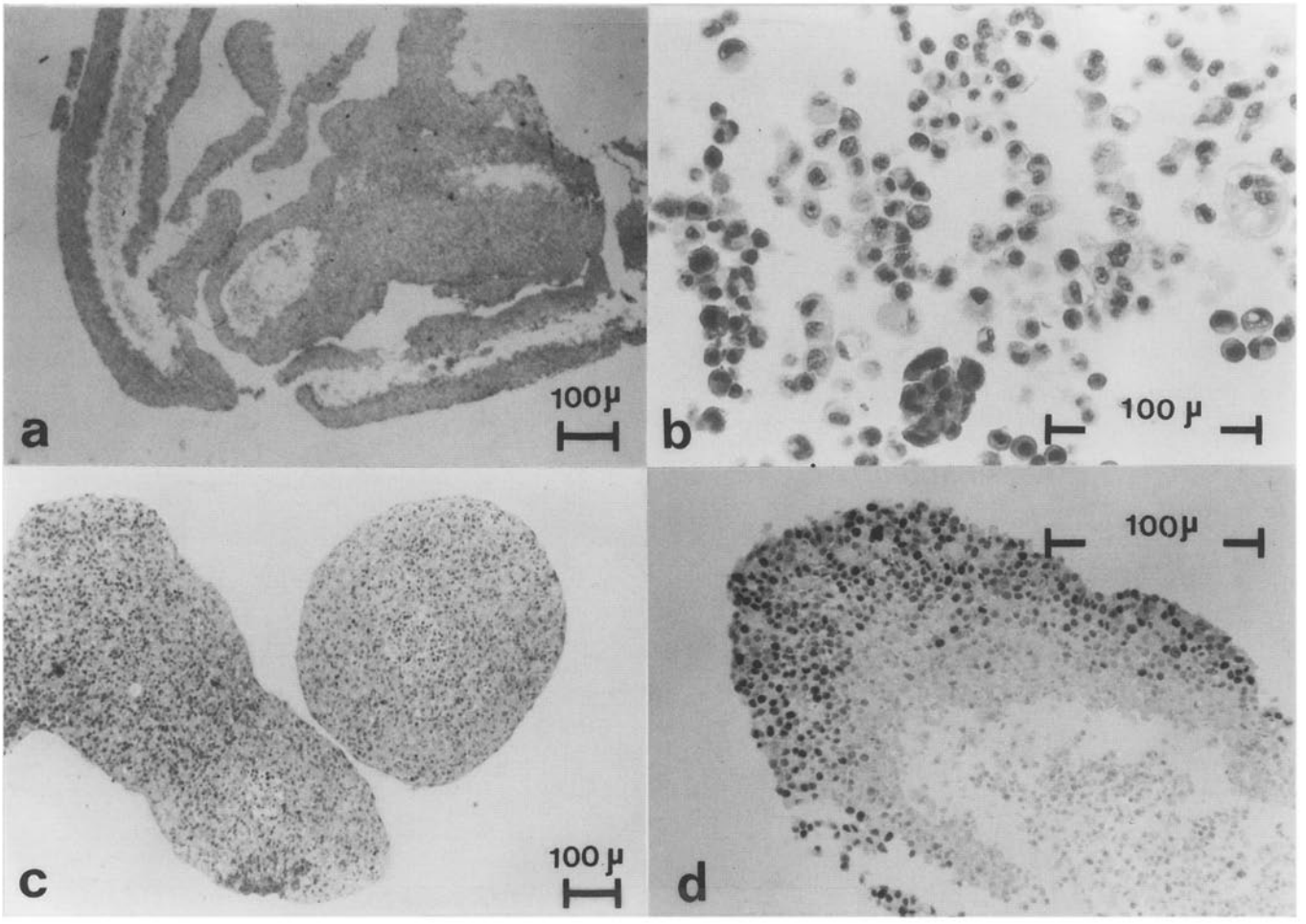

FIG. 3. Three-dimensional growth of three prostate cancer cell lines: (*a*) LNCaP, (*b*) PC3, (*c*) DU145, (*d*) Proliferating cell nuclear antigen (PCNA) staining of LNCaP spheroid showing more intensive staining in the peripheral zone. Photographed at ×100 (*a* and *c*), ×400 (*b* and *d*).

times only lightly tethered to the spheroid, reminiscent of the way in which mitotic cells in monolayer cultures detach. This spatial distribution of mitotic cells was confirmed when sections of spheroids were stained to demonstrate proliferating cell nuclear antigen (Fig. 3 *d*). Cells lost from superficial layers or from fractured spheroids were available to form new spheroids, a phenomenon that probably accounts for the occasional presence of small "new" spheroids in some older cultures.

When sections of spheroids were compared with sections of centrifuged pellets of monolayer-grown cells, it was clear that spheroids were not merely randomly aggregated cells. Rather, they showed tissue-like patterns that were reproducible for a given cell line. Cells within the spheroid conformed to each other and to groups of neighboring cells so that there were sometimes whorl-like patterns, areas of acellular matrix deposition, and other tissue-like characteristics. Cell line-specific morphology is illustrated in Fig. 4 in which the characteristic morphology of spheroids of PC3, LNCaP, HBL2, and NPF 209 can be compared with centrifuged pellets of monolayer-grown cells. As observed by others working with spheroids grown in static culture, cells in the central zone of larger spheroids cease proliferating and, with time, increasing percentages of these cells contain pyknotic or fragmented nuclei. Sutherland and others have shown, however, that some cells in the central zone of spheroids in static cultures remain viable and capable of repopulating the proliferating compartment (2,8,23). Similar observations have been made on cells from the necrotic, avascular areas of tumor nodules *in vivo* (19).

Scanning electron microscopy (SEM). The SEM morphology of cells in true spheroids was broadly similar with respect to gross morphology and exhibition of tissue-like architecture and cell surface features. Fig. 5 is representative of HBR 84 glioma cells grown under anchorage-dependent and microgravity conditions.

Confluent HBR 84 glioma cells grown under anchorage-dependent conditions were for the most part featureless (Fig. 5 *a,c*). The cells tended to overlap and grow on top of each other but were generally smooth and devoid of surface complexity. Some cells showed a few microvilli and may represent recently dividing cells. Such microvilli-containing cells were most frequent in cells visualized from subcon-

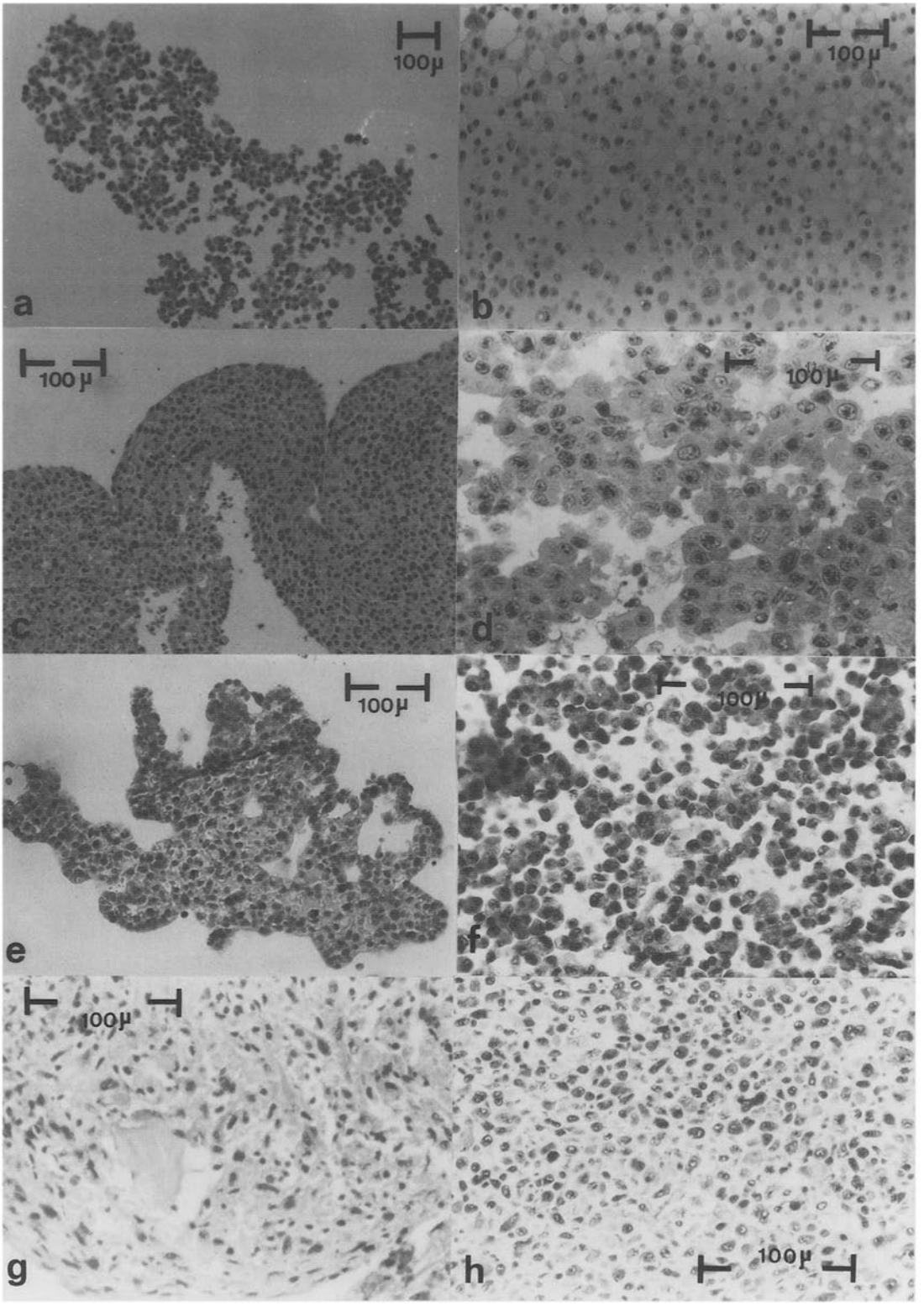

FIG. 4. Tissue-like pattern of four cell lines grown as spheroids in the HARV bioreactor (*left column*) contrasted with corresponding centrifuged pellet of monolayer-grown cells (*right column*). (*a* and *b*) = PC3: (*c* and *d*) = LNCaP: (*e* and *f*) = HBL2: (*g* and *h*) NPF 209. Photographed at ×100 (*a*); ×200 (*b,c,e*) and ×400 (*d,f,g,h*).

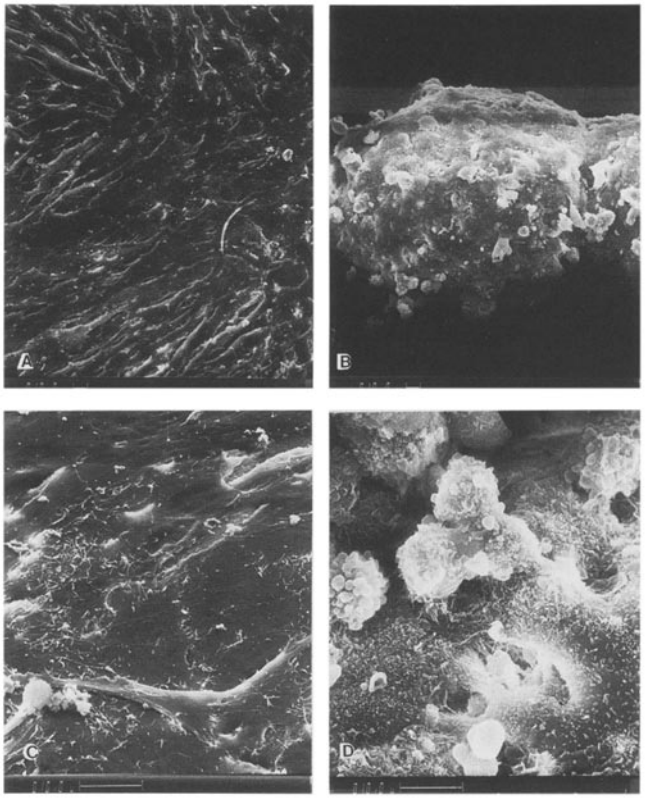

FIG. 5. Scanning electron micrograph of the glioma cell line HBR 84 grown as monolayer (*a* and *c*) and as spheroids in the NASA HARV bioreactor culture vessel (*b* and *d*). Original magnifications: (*a* and *c*) ×300, (*b* and *d*) ×1500. The micrometer markers of 10 and 100 μm in the micrographs indicate the actual magnification.

TABLE 3

ANTIGEN EXPRESSION

Cell Line	Cytokeratin	Antigen CD44	E Cadherin
HBL_2 bladder cancer			
Monolayer	3–4+	1+	±
Whole tissue	3+	1+	
Spheroid (14 d)	4+	2–3+	3+
$J8_2$ bladder cancer			
Monolayer		1–3+	1+
Spheroid (21 d)		3–4+	2–3+
C_3 prostate cancer			
Monolayer	2–3+	2–3+?	0–1+
Spheroid (14 d)	4+	3–4+	1–2+
DU 145 prostate cancer			
Monolayer	2+		
Spheroid	2+		
LNCaP prostate cancer			
Monolayer	2+	±	1+
Spheroid (1 d)		1+	1+
Spheroid (21 d)	2+	3–4+	3–4+
Spheroid (28 d)		3+	3–4+
HBR84 glioma (monolayer)		neg	
Spheroid (4 d)		+	
Spheroid (7 d)		3–4+	

fluent, rapidly growing cultures which also had rounded (mitotic) cells with numerous microvilli (data not shown). Spheroids of HBR 84 cells obtained from microgravity culture conditions, however, appeared as tightly bound masses of cells, almost tissue-like in their overall appearance (Fig. 5 *b,d*). Almost all cells visualized showed complex cell surfaces composed of blebs, ruffles, and microvilli. Their overall organization and morphology was more reminiscent of tumor cells *in vivo* (Fig. 5 *b,d*).

Cell interactions. The cellular interactions that occurred among like cells in the HARV bioreactor resulted in more than the conformational and shape changes illustrated in Figs. 3 and 4. Immunohistochemical staining showed that in producing three-dimensional structures, the cells also tended to upregulate the expression of the cell adhesion molecules, E cadherin and CD44 (Table 3). Increased expression of the epithelial cadherin molecule was demonstrable as early as the first day in bioreactor cultures. The cell adhesion molecule CD44 is a hyaluronan receptor that includes a heterogeneous group of cell surface glycoproteins that are expressed on leukocytes and metastasizing tumor cells. These cell adhesion molecules participate in regulating cell-to-cell and cell–substrate interactions and in cell migration (17,21).

Various differentiation markers were expressed by cells in monolayer cultures as well as in spheroids. For example, glial fibrillary acidic protein (GFAP) was expressed relatively uniformly and intensely by HBR 147 glioma cells cultured in both modes. Similarly, cytokeratin VIII was expressed by epithelial cell lines and collagen IV by fibroblast cell lines cultured either in monolayer or in the bioreactor. Immunostaining with cytokeratin VIII and collagen IV was especially useful when we observed the interaction of epithelial cells and fibroblasts in cocultures. By the fourth day in culture, normal prostate fibroblasts (NPF 209) produced compact spheroids approximately 0.5 mm in diameter that stained intensely throughout for collagen IV. PC3 cells did not express collagen IV but stained intensely with the epithelial marker, cytokeratin VIII. When a suspension of PC3 cells was added to the 4-d spheroids of fibroblasts, the PC3 cells tended to coat the spheroids not merely as loosely adherent cells but as a tightly compacted mass of flattened cells. This was in distinct contrast to the morphology of PC3 cells when cultured alone in either culture mode. Even in centrifuged pellets of monolayer-grown cells, the PC3 cells tended to resist compaction and flattening. Along with this prominent change in configuration, some of the PC3 cells penetrated the fibroblast spheroids and sometimes replaced part of the spheroid (Fig. 6).

The profound influence of epithelial cells on fibroblasts is especially well illustrated in the cocultures that were stained to demonstrate the presence of tenascin, an extracellular matrix glycoprotein produced by embryonic mesenchyme but not by fibroblasts in fully differentiated tissue. It has been demonstrated that malignant epithelium, like epithelium in the embryo and during wound healing, induces the production of tenascin by the underlying fibroblasts (6). Tenascin, in turn, may stimulate growth of the epithelium. Recently it has also been shown that tenascin is a general immunosuppressive molecule that interferes with T cell receptor/CD3-dependent signal transduction in T-cell activation (13). The coculture described above and other cocultures not reported here showed that when malignant epithelial cells, in this case PC3, attach to normal fibroblasts, they did indeed induce the expression of tenascin (Fig. 6 *c,d*). Tenascin expression is not immunohistochemically detectable in normal fibroblasts not in contact with malignant epithelial cells.

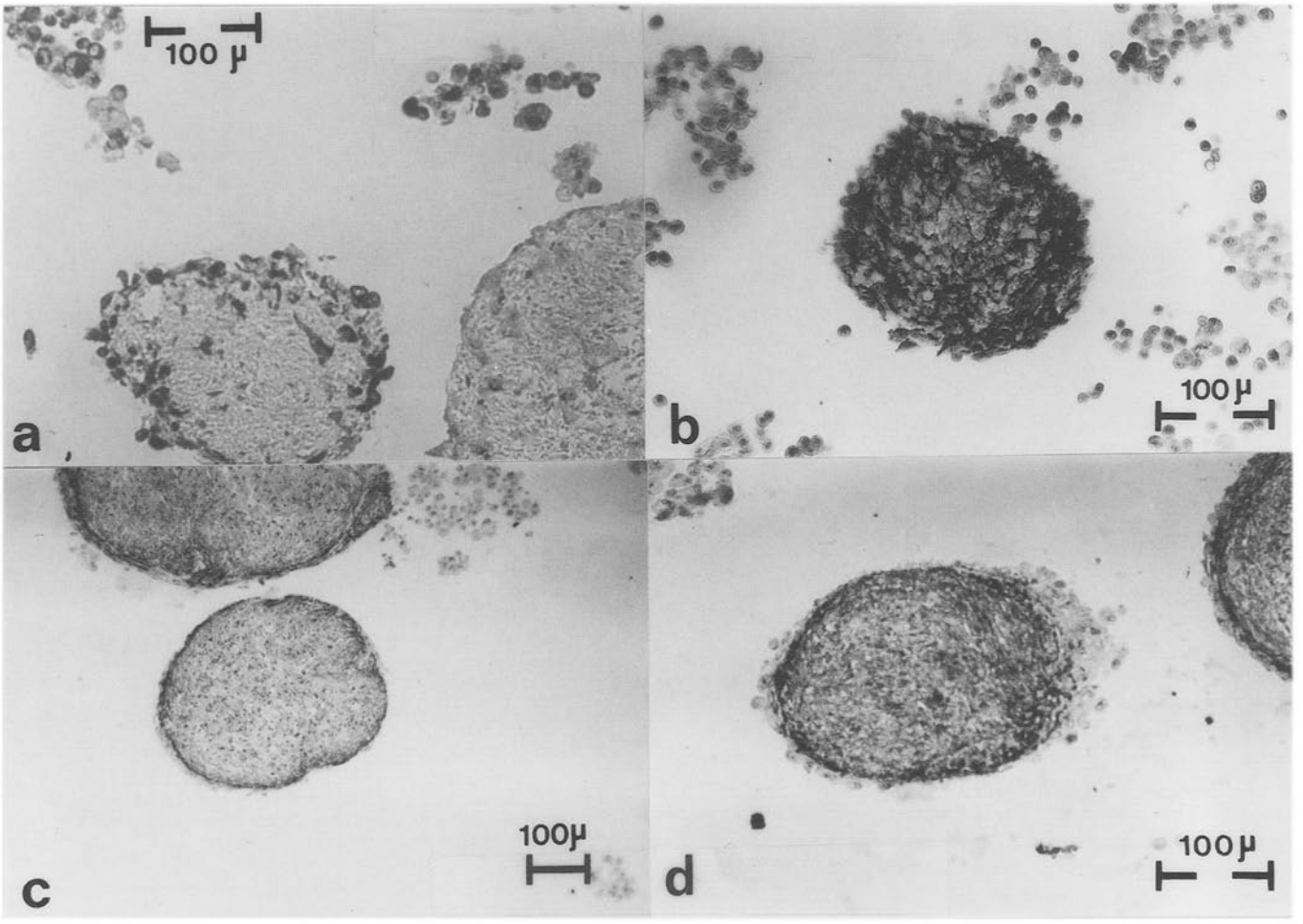

FIG. 6. Immunohistochemical staining of prostate cancer cells PC3 with preformed spheroids of fibroblasts. All sections are lightly counterstained with hematoxylin (*a*) PC3 cells, free-floating as well as those attached to and penetrating the fibroblast spheroid are cytokeratin VIII positive; (*b*) intense collagen IV reaction of fibroblasts; negative in PC3; (*c*) fibroblasts underlying the thin surface coat of PC3 cells demonstrate presence of tenascin; (*d*) more intense tenascin staining under the cap of epithelial cells. Photographed at ×100 (*c*) ×200 (*a,b,d*)

DISCUSSION

The considerable variety of methods developed for generating three-dimensional cellular constructs *in vitro* testifies that such cultures are useful and provide information not readily attained through other culture methods. Although the low shear, HARV culture system described earlier was developed to mimic microgravity, it is proving advantageous in a variety of studies that are by no means limited to the biological effects of space flight. Applications include tissue engineering (e.g., cultivation of cartilage on polymer scaffolding for use in prostheses), growth and differentiation of several types of human cancer cells, and tissue modeling generally (3,9,10,20). In many of these studies, cells have been cultured on carrier beads. Although the use of such cell support is advantageous in some studies, carrier beads are not essential for culture in the HARV bioreactors. Even cells that are otherwise considered to be anchorage dependent adapt readily to the bioreactor conditions. Initially we attempted to quantify the yield of cells from the bioreactor cultures but this proved to be unrewarding. Varying proportions of the cell populations attach to the bioreactor walls and some cells growing in spheroids are damaged or destroyed when more aggressive dispersal techniques are required. In a few cases, nearly as many cells were attached to the chamber walls as were growing in spheroids. Because the quantitative studies were not the primary goal, they were not pursued extensively and are mentioned here simply to alert investigators concerned with quantifying cell yields that they may need to use special procedures for such analyses. The other limitation encountered in the bioreactor studies reported here is difficulty in establishing primary cultures starting from minced tumor specimens. So far it has been necessary to establish a primary culture in a standard tissue culture flask and then use the early passage cells to seed the bioreactor culture. Other investigators, however, have been successful in establishing primary cultures in rotating wall bioreactors using carrier beads to provide anchorage for the tissue mince (10).

Perhaps the most intriguing capability conferred by the rotating, low-shear bioreactor system is the opportunity to study, under controlled conditions, the interaction of cells of a given type or the interaction of one cell type with another when cells in suspension are free to form their own associations without being separated by hydrodynamic forces within the culture. Comparison of cells in monolayer culture, monolayer-grown cells that have been centrifuged into a pellet and cells that have spontaneously formed spheroids in the bioreactor shows that the formation of three-dimensional cell constructs has a profound influence on cell shape and configuration. Gene expression is also affected by three-dimensional growth as illustrated by the increased production of the cell adhesion molecules, E cadherin and CD44, by cells in spheroids. Recently it was suggested that expression of specific CD44 splice variants might be restricted to tumors and thus clinically useful as a diagnostic tool (15). A subsequent rigorous test of this hypothesis, however, with exon-specific monoclonal antibodies showed that the putative tumor-specific splice variants were not always present in CD44+ tumors and furthermore that they were often present in normal tissues (7). Nevertheless, expression of variant CD44 by tumor cells is still considered to play a role in tumor metastasis (5).

The HARV bioreactor culture system provides a convenient experimental method for manipulating the proportional representation of different cell types in coculture or culturing a dispersed cell suspension of one type with preformed spheroids of another cell type, or for a type of confrontational culture as described by Laerum and others in their extensive studies on the interaction of malignant gliomas with normal brain tissue in static cultures (14). Coculture of the prostate cancer cell line, PC3, with normal fibroblast spheroids reported here is another example of confrontational cultures. It was the first in an ongoing series of experiments concerned with interactions of malignant epithelium and stroma in tissue-like constructs. This experiment and others in progress demonstrate that the low-shear suspension culture system may be useful for assaying invasive properties of cells such as malignant epithelium, endothelium, activated lymphocytes, and other cells of interest. The low-shear, rotating bioreactor provides considerable flexibility in establishing cocultures for studying cellular induction phenomena such as tenascin production. Results of the interactions may be apparent within a few days. Medium is relatively abundant and easily sampled or supplemented, an advantage when studies require assays for cells products, such as tenascin, bioactive peptides, growth factors, and so forth.

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