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Prospects for Use of Microgravity-Based Bioreactors to Study Three-Dimensional Host–Tumor Interactions in Human Neoplasia

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Abstract Microgravity offers unique advantages for the cultivation of mammalian tissues because the lack of gravity-induced sedimentation supports three-dimensional growth in batch culture in aqueous medium. Bioreactors that simulate microgravity but operate in unit gravity provide conditions that permit human epithelial cells to grow to densities approaching 10^7 cells/ml on microcarriers in suspension, in masses up to 1 cm in diameter, and under conditions of low shear stress. While useful for many different applications in tissue culture, this culture system is especially useful for the analysis of the microenvironment in which host matrix and cells interact with infiltrating tumor cells. Growth in the microgravity-based bioreactor has supported morphological differentiation of human colon carcinoma cells when cultured with normal human stromal cells. Furthermore, these co-cultures produced factors that stimulated goblet cell production in normal colon cells in an in vivo bioassay. Early experiments also suggest that the microgravity environment will not alter the ability of epithelial cells to recognize and associate with each other and with constituents of basement membrane and extracellular matrix. These findings suggest that cells grown in bioreactors that simulate aspects of microgravity or under actual microgravity conditions will produce tissues and substances in sufficient quantity and at high enough concentration to promote characterization of molecules that control differentiation and neoplastic transformation.

Key words: microgravity-based bioreactors, three-dimensional host–tumor interactions, batch culture, epithelial cells, neoplastic transformation

Bioreactors have generally been used for the large scale production of microorganisms, cells, or their products. Biotechnology requires large quantities of cells to isolate molecules that are often in small concentration and not secreted into the medium. Current technologies for batch culture of cells have been well described elsewhere [Miller et al., 1989; Ramasubramanyan and Venkatasubramanian, 1990] and are beyond the scope of this review. However, a bioreactor that is based on principles derived from research in microgravity may be valuable in the research laboratory for the production of cells in three-dimensional structures that are large enough to facilitate molecular characterization. Microgravity-based bioreactors may be used to probe aspects of cell biology that are difficult to define either in monolayer culture, embedded gel, or hollow fiber systems now available in the basic research laboratory. Microgravity-based bioreactors also may be suitable for large scale batch culture of mammalian cells. Our purpose in this review is to assess the use of a small-scale microgravity-based bioreactor operated in unit gravity on earth to analyze the interaction between human colon carcinoma cells and its host. We will describe the potential that such bioreactors may have for tissue formation when they operate in actual microgravity. The focus of our research is to characterize the molecular interactions between a neoplasm and its host to define molecules that may inhibit tumor growth. We will demonstrate how a microgravity-based bioreactor may aid in the analysis of complex tissue interactions with this problem as a paradigm for the cultivation of cells in a high fidelity three-dimensional architecture.

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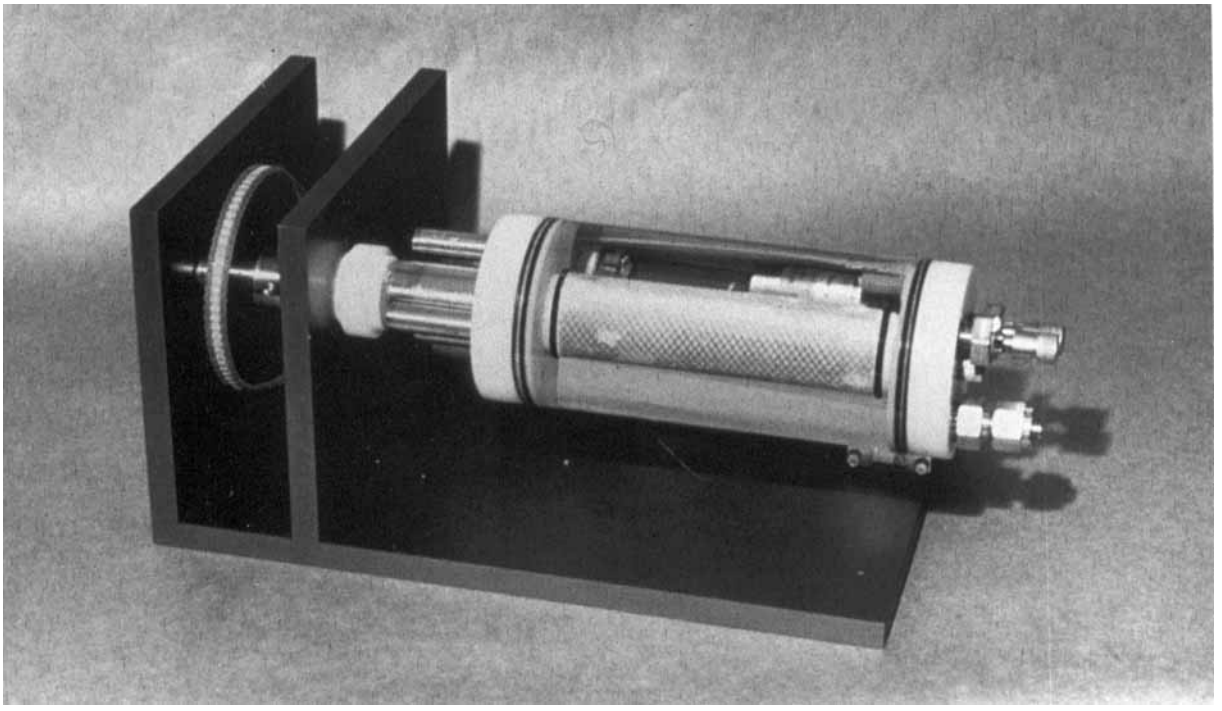

Fig. 1. The rotating wall vessel (RWV) is the microgravity-based bioreactor. Medium with microspheres is placed in the cylinder which is then rotated around the horizontal axis.

THE MICROGRAVITY-BASED BIOREACTOR

The microgravity-based bioreactor was developed at NASA-Johnson Space Center and is a zero-head space, aqueous medium-filled clinostat that suspends particles by rotating the vessel wall and spin filter (hence, the term rotating wall vessel or RWV) around the horizontal axis (Fig. 1). In its simplest form, the RWV has a capacity of 125–500 ml with a central spin filter covered with a semipermeable membrane that permits gas diffusion. Nonadherent cells are cultured in suspension, while adherent cells are grown on microcarrier beads. Cells or beads are maintained in suspension by balancing their sedimentation induced by gravity with centrifugation caused by vessel rotation. Rotation of the RWV at speeds of 10–60 rpm maintains particles that are up to 1 cm in laminar streamlines [Tsao et al., 1991], so that individual particles behave as though they were in a continuous fluidized bed reactor. This also means that cells or particles are subjected to a randomized gravity field with low shear stresses. Conventional bioreactor systems create a shear stress of 3–10 dyn/cm^2 [Cherry and Papoutsakis, 1986]. Since cell viability is decreased at shear stresses greater than 5–7 dyn/cm^2 [Cherry and Papoutsakis, 1986, 1988], most culture systems operate in a high shear environment that restricts growth. The RWVs provide a suspension culture environment that initially has low shear stress, since individual microcarrier beads (150–175-μm diameter collagen-coated dextran beads) are subjected to stresses of 0.81 dyn/cm^2 [Tsao et al., 1991]. However, when the microcarrier beads are covered with cells, cells and beads raft together and form dense cellular packs of 5–50 beads that are 0.7–1.5 cm in diameter. Vessel wall rotation must be continuously increased to counteract gravity-induced sedimentation. Eventually, as particles attain 1.0-cm diameter, the shear stress of gravity-induced sedimentation restrict further growth in unit gravity. Nonetheless, in unit gravity the microgravity-based bioreactor supports the cultivation on microcarriers of masses up to 1 cm in diameter and cell concentrations of at least 10^7 cells/ml.

ALTERNATIVES FOR THE STUDY OF COLONIC TISSUES IN VITRO

Several alternative culture systems are available in the research setting for the analysis of tumor-host interactions: static cell culture, static matrix cultures, roller bottles, stirred suspen-

TABLE I. Characteristics of Different Research Bioreactors

Reactor Vessel	Advantages	Disadvantages
Static cell culture	No shear stress, easy product isolation, clear morphologic analysis	Low cell density, only two-dimensional with interaction of 2 monolayers
Static matrix cultures	Three-dimensional cultures, no shear stress, permits analysis of morphology	Diffusion limited cell densities, product isolation difficult
Roller bottles	Relatively high cell density, product isolation easy, high shear stress	Two-dimensional with monolayers, can be used to support microcarriers but higher shear stress than microgravity-based bioreactor
Stirred suspension culture	Cell density of 10^6–10^7 cells/ml with microcarrier beads	Low–moderate shear stress, two-dimensional system
Airlift bioreactors	Cell density of 10^6–10^8 cells/ml depending on cell type, moderate shear stress	Cells are encapsulated, making cell recovery difficult
Hollow fiber-perfused system	High cell density of 10^7–10^8 cells/ml, easy product recovery, three-dimensional growth feasible, no shear stress on cells	Diffusion limited along length of fiber and through membrane into cell mass, recovery of cells may be very difficult
Microgravity-based bioreactor RWV	Low shear stress, high cell density (at least 10^7 cells/ml), three-dimensional cultures, easy product isolation	Cell culture limited to masses of 1 cm in unit gravity

sion culture, airlift bioreactors, and hollow fiber perfused cell systems are among the most commonly used systems (Table I). Each of these systems has advantages and disadvantages for the analysis of the host-tumor environment. Static cell cultures are the simplest culture systems, do not have any shear stress, allow careful morphologic analysis of the interaction of two monolayers of cells, and relatively easy isolation of products secreted into the environment. The disadvantage of the static cell culture system is that it is essentially two-dimensional because cells grow as monolayers on flat plastic or plastic-coated surfaces and the cell concentrations are limited to $0.3–1 \times 10^6$ cells/ml. Static matrix cultures such as those created with Matrigel [Kleinman et al., 1982] by Hall et al. [1982] are very useful for growing cells in a true three-dimensional architecture that permits morphologic analysis of cells. However, cell densities in a gel matrix culture are severely limited by diffusion of nutrients and wastes through the matrix. This limits the number of cells from which novel molecules may be isolated as well as hindering the isolation of substances that are secreted into the microenvironment. Roller bottle cultures are excellent for growing monolayers of cells or cells on microcarriers but have not been exploited for assessment of cell interactions. Stirred suspension cultures and airlift bioreactors allow for the growth of cells to cell concentrations of 10^7–10^8 cells/ml under conditions of fluid mixing that requires some shear stress. These systems are usually used for the production of factors in batch cultures [Ray et al., 1990; Dean et al., 1987], and not used to study the interaction of different cell types. Finally, the most successful batch culture system available for the research laboratory is the perfused hollow fiber system in which cells are grown on one side of a semipermeable membrane while medium is perfused on the other side. This system has no shear stress and allows easy recovery of secreted products. The disadvantage is that diffusion of nutrients and wastes limits both the length of the fiber that can be perfused and the thickness of the cell mass that surrounds the fiber [Tharakan et al., 1988; Prokop and Rosenberg, 1989]. Nonetheless, the hollow fiber system allows for the three-dimensional culture of cells in the interstices between fibers [Rutzky et al., 1979].

The microgravity-based bioreactor compares favorably with these systems because it combines the favorable aspects of several of the other bioreactors. Since it uses microcarriers, it has a high surface area/volume ratio that facilitates nutrient and waste transfer. While the RWV is operated in a batch mode, secreted products may be isolated from spent medium more easily than can be done in matrix-based culture systems. While not as low as in a static system, the shear stress for particles that are less than 1 cm in diameter is quite low (0.92 dyn/cm^2) and less than the shear stress experienced in conventional stirred reactors. Furthermore, by using

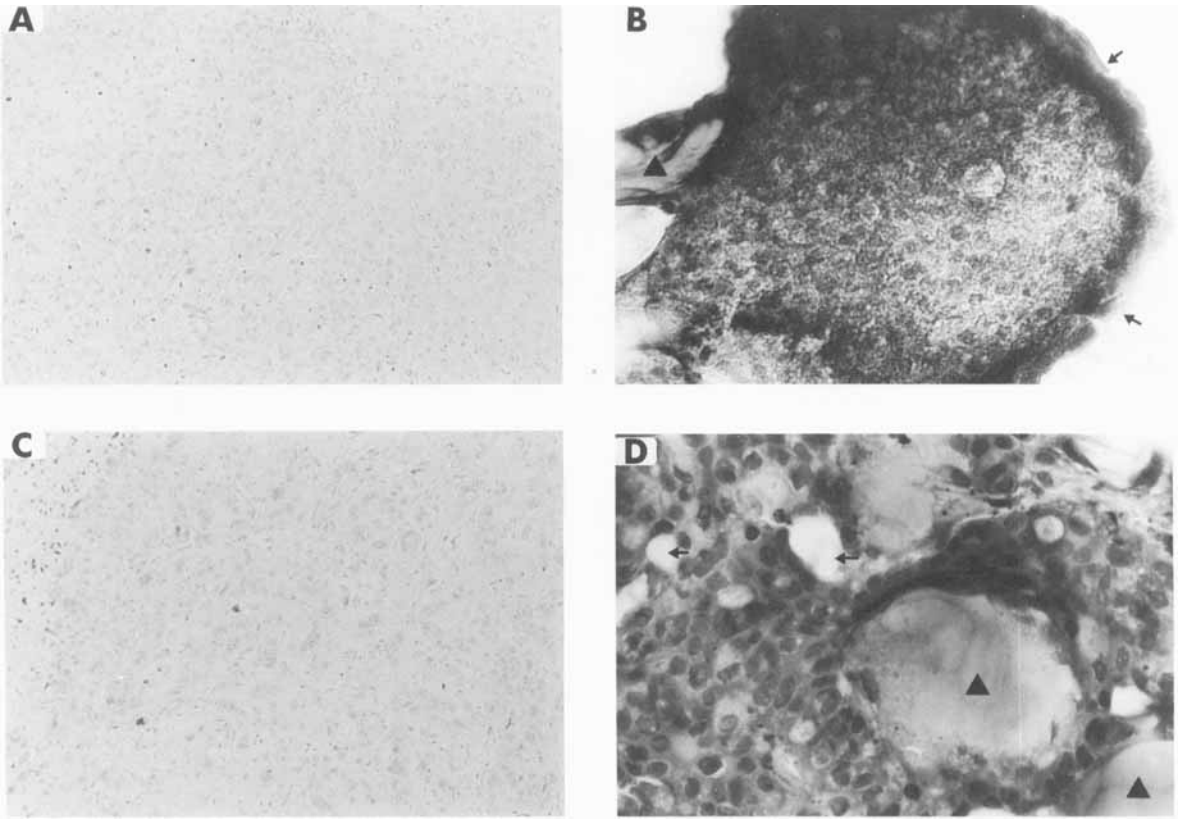

Fig. 2. Histologic comparison of mouse xenografts of HT-29 and HT-29KM vs HT-29 and HT29KM co-cultivated in RWVs, 5 μm H&E-stained cross sections of HT-29 (**A**) and HT-29KM (**C**) mouse xenografts, ×100, HT-29 shows no differentiation and HT-29KM shows areas of limited cellular differentiation and signet ring cell development (lower left), 10 μm H&E stained co-cultures of HT-29(B) and HT-29KM(D), ×200 **B:** A large polypoid growing out from the microcarrier (far left, triangle) Arrows mark the apex of the polypoid D, signet ring cell formations **D:** In contrast to C, this tissue shows structural organization and differentiation Multiple signet ring cells and glandular formation are visible In addition, a line of columnar epithelial cells can be seen to the immediate left of the microcarrier in the center of the photograph (D). [From Goodwin et al , 1992]

microcarriers, this type of bioreactor avoids encapsulating cells within calcium alginate beads that may prevent subsequent growth. The perfused hollow fiber system provides a good alternative to the RWV but the perfusion circuit may be more difficult to maintain than the simple batch operations in the RWV. The only disadvantage of the RWV system in its 125-ml configuration is that cells with stringent requirements must be seeded at a high enough concentration to sustain growth and this may require a total of $\sim 3 \times 10^7$ cells for cultures that require $2–3 \times 10^5$ cells/ml for initiation. Finally, while microcarrier systems are essentially two-dimensional spherical surfaces, the RWV allows for three-dimensional cultures since microcarrier beads may be rafted or aggregated by the cells growing on them. This permits anchorage-dependent cells to grow in three dimensions and in multiple layers within the framework provided by several microbeads crosslinked together. Since the framework is open to aqueous medium, nutrient transfer is improved over that in hollow fiber systems where cells are severely limited in diffusion once there are more than three cell layers deposited on the hollow fiber membrane.

USES OF A MICROGRAVITY-BASED BIOREACTOR FOR HUMAN COLON CARCINOMA CULTURES

In our first evaluation of the RWV we compared the growth of HT-29, a human colon adenocarcinoma established as a cell line by Fogh [1975], and its subline HT-29KM in the bioreactor to implants of these two cell lines in athymic nude mice [Goodwin et al., 1992]. HT-29 is poorly differentiated both in nude mice (Fig. 2) and when grown alone in the bioreactor (Fig.

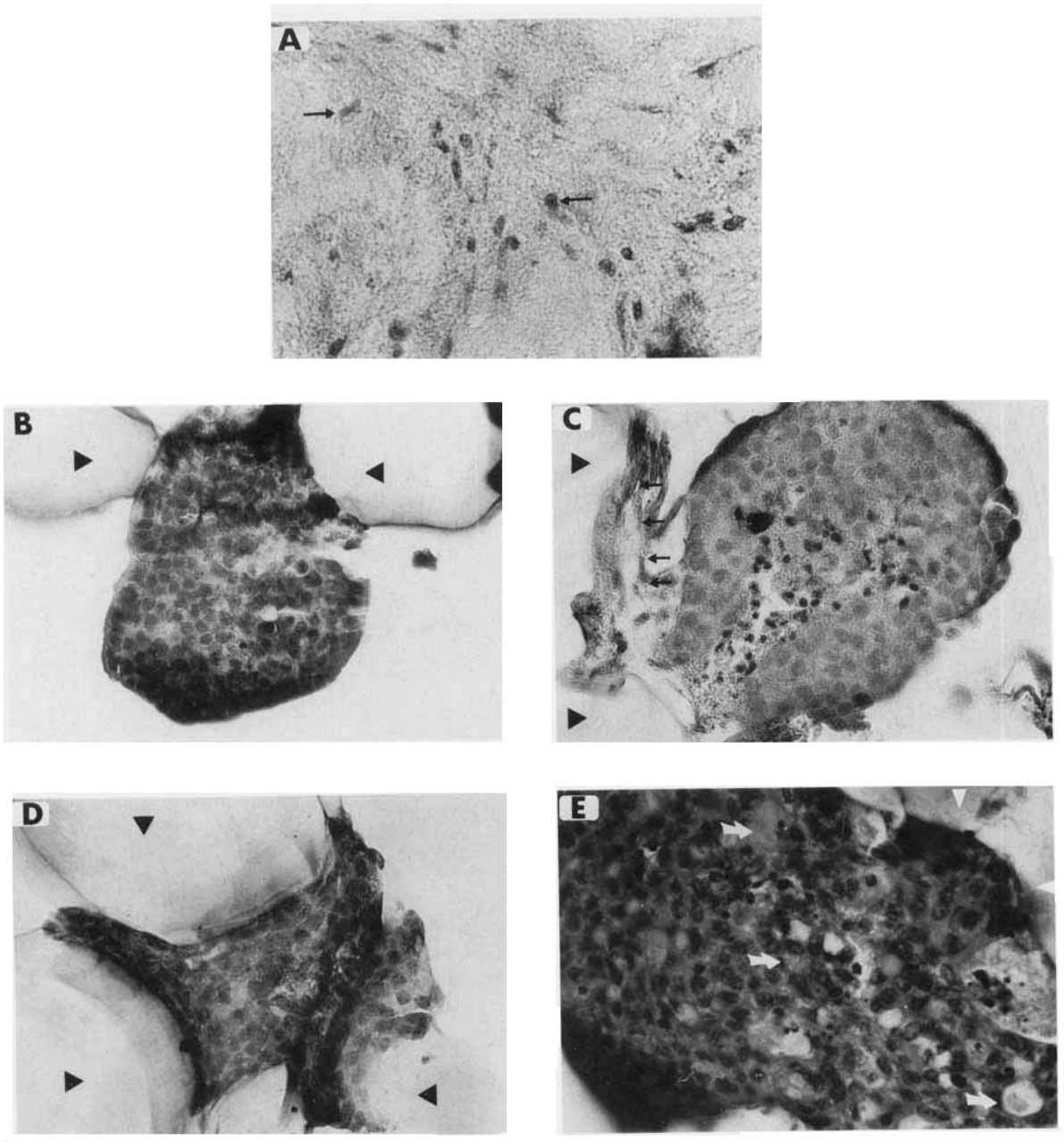

Fig. 3. Histologic comparison by mucicarmine of monocultured normal colon fibroblasts, HT-29 and HT-29KM vs co-culture in RWVs, 10-μm mucicarmine-stained cross sections of microcarrier bead packs (triangles) showing the normal colon fibroblasts (**A**), HT-29 (**B**), and HT-29KM (**D**) monoculture, ×200, 10-μm mucicarmine-stained cross sections of HT-29 (**C**) and HT-29KM (**E**) co-cultures ×200 In contrast to the growth of HT-29 in monoculture (B), co-cultivation of HT-29 with normal colon fibroblasts results in polypoid structural development (C) which is similar to that seen in vivo Note fibroblasts (arrows) on the surface of microcarriers (triangles) In contrast to the undifferentiated growth of HT-29KM (D), many packets of mucin (arrows) (differentiated secretory product), cellular differentiation, and organization can be seen in HT-29KM co-cultures (E) [From Goodwin et al , 1992]

3) or co-cultured with human colonic fibroblasts in the bioreactor (Fig. 2). The subline HT-29KM is also poorly differentiated when cultured alone in the bioreactor but differentiates as a xenograft in nude mice producing glands and mucin-filled signet ring cells (Fig. 2). When co-cultured in the bioreactor with human colonic fibroblasts, HT-29KM was induced to produce signet ring cells with mucin (Fig. 4) as well as glands (Fig. 2). Interestingly, when incubated with sodium butyrate or dimethyl sulfoxide, HT-29KM cells did not produce signet ring cells in stan-

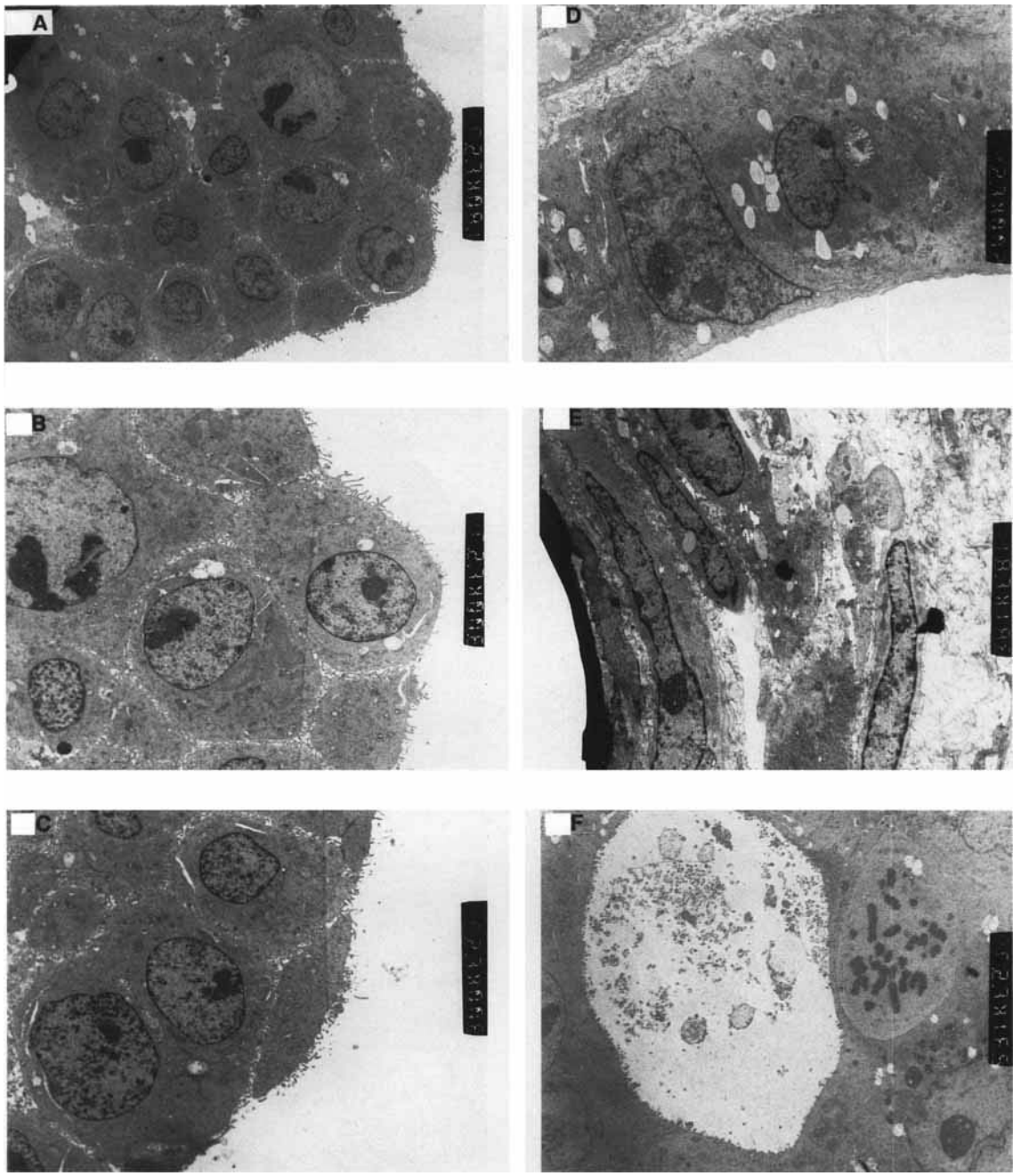

Fig 4 TEMs of HT 29KM in RWV co culture HT 29KM co cultivated with normal colon fibroblasts ×5 400 (**A**) and ×9 000 (**B,C**) Note the regularity of the nuclei and reduction in multiple nucleoli as compared to **D** and **E** Note the presence of an apical microvillus border (A C) and intercellular gland structures (**F**) HT 29KM co cultured with normal colon fibroblasts ×15 000 (D) ×103 000 (E) and ×7 000 (F) Note the columnar epithelium tight cellular junctions and sinusoid formation in D Normal colon fibroblasts layered over a microcarrier and large amounts of extracellular matrix produced in co culture (E) F interglandular structure formed by the junction of several cell borders with internal microvilli [From Goodwin et al 1992]

dard monolayer cultures (data not shown) Attempts to induce mucin production in HT-29KM cells by glucose deprivation of confluent monolayers also failed in standard monolayer cultures Since HT-29KM failed to differentiate when cultured by itself in the RWV, the interaction of HT-29KM cells with human colonic fibroblasts in the co-culture induced the differentiation of the neoplastic cells Kedinger et al [1987, 1987a] have shown that the interaction of mes-

enchyme and epithelium is important for the formation of basement membranes and for the morphological state of differentiation of normal colonic epithelial cells. Richman and Bodmer [1988] demonstrated that three-dimensional cultures in appropriate substrates were most conducive to induction of differentiation and glandular formation by several human neoplastic colon cell lines. Richman and Bodmer [1988] also found that HT-29 did not form glands or produce signet ring cells when embedded in matrigel. Fukamachi et al. [1987] have observed that HT-29 cells do not differentiate when embedded in a fetal rat intestinal mesenchyme in a static gel matrix culture. Our data support the conclusion of these investigators. In addition, Bouziges et al. [1991] observed that HT-29 cells interacting with skin fibroblasts do not produce the matrix components necessary to form basement membranes. Similar observations were made in our system because human colonic fibroblasts did not secrete extracellular matrix when co-cultured with HT-29 but did when co-cultured with HT-29KM (Fig. 4). Since the colonic fibroblasts were harvested from similar pools of three primary lines established from donors who had had colonic resections for non-neoplastic disease, the induction of the extracellular matrix was stimulated by the neoplastic cells interacting with the same set of normal colonic fibroblasts. Further, there was evidence that the epithelial cells and fibroblasts established polarity with the fibroblasts close to the microcarrier surface and the epithelial cells further away and oriented in columnar arrays (Fig. 4). These results with HT-29 and its differentatable subline HT-29KM indicate that the microgravity-based bioreactor supports cell interactions that confirm findings observed in static gel matrix cultures as well as inducing structures that had previously only been observed in xenografts in vivo.

RWV CULTURE INDUCES CEA EXPRESSION IN A POORLY DIFFERENTIATED CARCINOMA

The induction of differentiation in three-dimensional cultures is demonstrated with the MIP-101 cell line that fails to differentiate in monolayer-based systems even in the presence of potent differentiation inducers. MIP-101 was established in culture from malignant cells harvested from the abdominal fluid of a patient with colon cancer that had disseminated to the liver, lungs, and peritoneum [Niles et al., 1987]. MIP-101 does not produce CEA in culture. It forms tumors in athymic nude mice when implanted in the subcutis of the flank, the spleen, or the abdominal cavity [Wagner et al., 1990]. MIP-101 produces carcinoembryonic antigen (CEA, a glycoprotein involved in intercellular adhesion that is a clinically useful tumor marker) when it grows in the abdominal cavity (its site of origin) but does not produce CEA when implanted in the spleen or the subcutaneous tissue. MIP-101 is weakly metastatic in nude mice when cells are taken from standard monolayer tissue cultures and injected intravenously or intrasplenically to produce experimental lung or liver metastases [Wagner et al., 1990]. It does not produce spontaneous metastases when implanted in the flank. However, when it is implanted in the abdominal cavity and produces CEA, it produces spontaneous metastases in the lungs or liver of athymic nude mice [Wagner et al., 1990]. Furthermore, nude mice that are pretreated with CEA intravenously followed by an intrasplenic injection of MIP-101 cells from standard tissue culture not only form experimental liver metastases but also experimental lung metastases [Wagner et al., 1990; Jessup, submitted].

These results suggest that (1) the production of CEA is important for MIP-101 cells to metastasize to other sites and (2) the production of CEA by MIP-101 cells is induced by the microenvironment of the abdominal cavity of the athymic nude mouse. In this view, the environments provided by the subcutaneous tissue or the spleen inhibit the production of CEA by MIP-101 cells, whereas some factor or condition in the abdominal cavity stimulates CEA expression. The induction of CEA expression then forms a test to analyze the effect of a three-dimensional environment upon MIP-101 cells. When MIP-101 cells are cultured in monolayer culture on various substrates (including Matrigel), CEA secretion was not stimulated (Table II). Even the incubation of MIP-101 cells with 2 mM sodium butyrate or DMSO for five days did not induce the production of CEA (Table II). No mRNA for CEA was identified in MIP-101 by polymerase chain reaction (data not shown). However, when MIP-101 cells were cultured in the RWV for five to seven days, they attained a concentration of $\sim 4 \times 10^6$ cells/ml and formed 3–4 mm spheroids that produced CEA (Table II). This induction of CEA expression in the RWV provides a new interpretation for the ef-

TABLE II. MIP-101 Cells Grown in the RWV Produce CEA[a]

Treatment	Cells/ml ($\times 10^6$)	Type of culture	CEA ($ng/10^6$ cells/day)
None	0.13–1.30	Monolayer	0.0–0.95
Matrigel	0.13	Monolayer	0.5
Laminin	0.10	Monolayer	0.0
Butyrate (2 mM)	1.70	Monolayer	0.09
DMSO (1.25%)	0.12	Monolayer	0.38
RWV	0.58	RWV	76.79

[a]Cells were plated at 2×10^4 cells/ml either in T25 flasks or in 35-mm Petri dishes (on laminin or Matrigel coated surfaces) in RPMI 1640 medium with 10% fetal calf serum. The RWV cultures were initiated with 2×10^5 cells/ml. After 5 days, the medium was harvested and tested for the presence of CEA by RIA. The number of cells present was enumerated following enzymatic dissociation into a single cell suspension.

fect of the host microenvironment upon the induction of CEA expression in this neoplastic cell line. Prior to the RWV experiments the interpretation would be that the peritoneum, as the orthotopic site from which the cells were harvested, stimulated the cells to produce CEA and that the subcutaneous tissue or the spleen inhibited the expression of CEA. In light of the RWV results, the spleen and subcutaneous tissue may inhibit CEA expression but the abdominal cavity does not stimulate CEA production. Instead, the abdominal cavity permits CEA production when the cells grow as three-dimensional spheroids. Work is in progress to determine whether the induction of the expression of CEA in the RWV cultures is associated with an increase in the metastatic potential of MIP-101 cells in nude mice. Since the induction of CEA expression in this cell line is associated with aggressive behavior, it is also important to define how three-dimensional cultures stimulate CEA expression and whether the induction of CEA expression produces a more metastatic phenotype. The static matrix gel system involving Matrigel or other preparations of extracellular matrix may confirm that cells cultured as spheroids in three-dimensional cultures make CEA. However, access to a batch system such as the RWV that provides nearly 10^9 cells and > 100 ml of conditioned medium will greatly facilitate analysis of the molecules involved in the induction of CEA expression.

POTENTIAL FOR THE MICROGRAVITY-BASED BIOREACTOR TO PRODUCE GROWTH FACTORS

The HT-29 cell line is capable of differentiating into absorptive [Huet et al., 1987] or mucin-filled goblet cells [Phillips et al., 1988] under appropriate stimuli. These pathways of differentiation are similar to those taken by cells that arise from cycling stem cells at the base of the crypt of Lieberkuhn, migrate up the crypt as they terminally differentiate, and are released into the lumen of the gut. The process that controls the maturation and terminal differentiation of normal colonic epithelium is poorly understood but may involve soluble factors. Since human colorectal carcinomas implanted in athymic nude mouse bowel induce mucosal hypertrophy and hyperplasia over the implant [Hostetter et al., 1988; Sekikawa et al., 1990], we sought to determine whether substances released from human colorectal cells cultured in vitro induced similar changes in normal mouse colon. Spent medium from various types of tissue cultures was encapsulated in liposomes that were then implanted into the cecum of nude mice. Media from RWV cultures of HT-29 and HT-29KM induced not only a proliferation in the total number of cells in the crypts of Lieberkuhn but also a small, but significant expansion in the relative and absolute number of goblet cells (Table III). When media from monolayer co-cultures were tested in this bioassay, they did not stimulate the expansion of the goblet cell compartment and had a minor hyperplastic effect (data not shown). Similarly, roller bottle co-cultures also lacked this selective effect on goblet cell proliferation and maturation but did stimulate a potent hyperplastic response (Table III). While the identity of the factor stimulating production of goblet cells is unknown, it may be a new effect of previously defined growth factors or a novel intestinal growth factor similar to mucomodulin [Irimura et al., 1990]. Purified gastrin and epidermal growth factor while trophic, did not significantly increase the proportion of goblet cells (data not shown). Nonetheless, the RWV operating in unit gravity provides a culture system that may enhance the isolation of growth factors. A better understanding of the molecules that control the maturation and proliferation of normal colonic epithelial cells is important to ameliorating inflammatory bowel disease, the gastrointestinal effects of chemother-

TABLE III. Effect of Goblet Cell Differentiation Factor (GCDF) Produced in the RWVs on Murine Colon Crypt Cells

	Treatment	Crypt height[a]	Crypt cell no.[b]	Crypt goblet cell no [c]	% Goblet cell[d]
Exp 1 (RWV)	Saline	33.6 ± 0.9	36.3 ± 1.1	3 0 ± 0.2	10.7 ± 0 8
	Medium	32.2 ± 0.8	35.3 ± 0.9	3.8 ± 0.2	10 9 ± 0.6
	HT-29 Medium	44.8 ± 0.9*	42.5 ± 1.3	5.7 ± 0.3*	13 6 ± 0 8*
Exp. 2 (RWV)	Saline	58.5 ± 1.3	28.7 ± 0.7	6 0 ± 0 2	21 2 ± 0.9
	Medium	64.4 ± 1.4	33.4 ± 1.3	6.5 ± 0.3	19.5 ± 0.6
	Fibroblasts	56.9 ± 1.0	30.3 ± 0.7	5.6 ± 0.3	18.5 ± 1 1
	HT-29KM/ Fibro Coculture	90.9 ± 1.7*	33.0 ± 1.4*	9.8 ± 0.6*	25 6 ± 1.4*
Exp 3 (Roller Bottle)	None	40.3 ± 1.0	20.2 ± 0.4	3.2 ± 0 2	15 8 ± 0 8
	Saline	66.2 ± 2.8	32.1 ± 1.5	5.1 ± 0.3	16 4 ± 1.1
	Medium	74.2 ± 2.1	33.1 ± 0.9	3 6 ± 0 2	11.0 ± 0 7
	HT-29 Medium	93.2 ± 2.3*	47 4 ± 1.0*	7.9 ± 0.3*	16 7 ± 0 7

*$P < 0$ 01 compared to saline controls by ANOVA and Scheffe F test

[a]Mean ±SEM of the height of sagittally sectioned crypts (in 1/250 mm) over submucosal liposomes

[b]Cell number is the mean ±SEM of the total number of cells in one-half of a sagittally sectioned crypt

[c]Goblet Cell Number is the mean ±SEM of the number of goblet cells per half crypt

[d]Percentage of Goblet Cells is the goblet cell number divided by the number of cells per half crypt for each individual crypt (Mean ±SEM)

0 15M phosphate-buffered saline (saline), tissue culture medium (alpha MEM with 10% fetal calf serum, supplemented with 2 ng/ml of EGF) or culture conditioned by the HT-29 colon carcinoma cell line cultured in the RWV was encapsulated into liposomes, 0 05 ml of 6 μM of liposomes in PBS was injected into the submucosa BALB/c mice The culture medium and supernatants had been concentrated 10-fold and one ml incorporated into 60 μM of CGP 19,835 A lipids manufactured by Ciba-Geigy Five days later, the mice were killed and the colons harvested, irrigated with saline, and each cecum fixed in formalin Paraffin sections were then stained with hematoxylin and eosin and the morphometric analysis was performed Forty crypts were counted per treatment group that was sagittally sectioned over the liposomes In Exps 1 and 2, the medium was harvested from cultures in the RWV, in Exp 3, the conditioned medium was collected from a roller bottle culture of HT-29 Particles in the roller bottle were less than 0 5 cm, while the RWV cultures contained many particles between 0 5 and 1 0 cm While all the media stimulated colonic crypt hypertrophy (increased crypt height) and hyperplasia (increased crypt cell number), only the RWV cultures in Exps 1 and 2 significantly expanded the percentage of goblet cells in the crypt Thus, only the RWVs allowed for production of a detectable amont of a goblet cell stimulating activity

apy, as well as providing a potentially new form of therapy for cancer.

MICROGRAVITY-BASED BIOREACTOR DOES NOT ALTER EPITHELIAL CELL ADHESION

If cells form masses in three-dimensions that accurately replicate either the structure or the function of tissue, then it may be possible that tissues can be engineered in microgravity. As described elsewhere in these Prospects, cartilage cells form cartilage on preformed biodegradable supports. This means that cartilage and, perhaps bone, can be grown to shapes designed by a computer to replace or supplant bone or cartilage that is missing either through a birth defect (e.g., craniofacial abnormalities) or through trauma. The design of more complex tissues such as organs will be infinitely more complicated. However, one of the first steps in the formation of tissue is for the individual cells to recognize each other and then associate appropriately. It is not clear that in microgravity cells will be able to adhere to either extra- or intercellular adhesion ligands. We started analysis of this problem by assessing whether cells cultured in the RWV for 5 or more days are capable of binding to standard extracellular matrix proteins such as laminin and fibronectin as well as the intercellular adhesion glycoprotein CEA. Cells cultured in simulated microgravity have the same capability of binding to adhesion ligands as do cells grown in standard monolayer cultures (Table IV). This suggests that cells will maintain their ability to sort and form struc-

TABLE IV. Culture in the RWV Does Not Inhibit the Ability of Epithelial Cells to Bind to Inter- and Extracellular Ligands[a]

Substrate to which cells adhere	% Cells bound (Mean ±SEM)					
	KM-12c		MIP-101		CCL 188	
	RWV	Control	RWV	Control	RWV	Control
None	6 ± 1	3 ± .1	14 ± 1	10 ± 1	20 ± 2	1 ± .1
CEA	32 ± 3*	6 ± 0.3*	13 ± 1	9 ± 1	31 ± 3*	16 ± 1*
Collagen IV	58 ± 2	26 ± 1*	29 ± 1*	61 ± 2*	47 ± 4*	54 ± 0.6*
Fibronectin	3 ± 1	4 ± 1	11 ± 1	11 ± .3	22 ± 2	1 ± 0.1
Laminin	57 ± 9*	19 ± 0.4*	53 ± 1*	63 ± 2*	25 ± 2	43 ± 4*

[a]Cells were started from the same stock and harvested from standard monolayer or RWV cultures after 6–7 days and analyzed for their ability to adhere to various ligands.

*Significant differences between the control (None) group and experimental groups of at least $P < 0.01$ by Scheffé test. While the magnitude of the adhesion to the various substrates may change with different culture conditions, the ability to bind is not impaired in the RWV culture. This suggests that cells will be able to associate in actual microgravity as well as they do in unit gravity.

tures in actual microgravity conditions and that microgravity may permit the creation of novel tissues.

POTENTIAL FOR CULTURE SYSTEMS OPERATING IN MICROGRAVITY

The microgravity-based bioreactor was designed to simulate certain aspects of microgravity. It was also designed to operate in a microgravity environment under process control. As of this writing, the bioreactor has flown short shuttle missions to determine whether the microcarriers will stay suspended as predicted when vessel wall rotation is stopped in microgravity. Confirmation of the lack of sedimentation in aqueous medium has recently been achieved for microcarriers in microgravity. This observation means that tissue cultures may be able to routinely form and replicate delicate three-dimensional structures. Should three-dimensional structures like the crypts of Lieberkuhn that line the mucosa of the large bowel be maintained in batch culture, the molecules that regulate proliferation and differentiation within these structures may be more easily identified and applied to situations in unit gravity. The characterization of such factors would be of tremendous medical and biologic benefit.

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