STEM CELLS

Volume 11, Number 2, 1993

To Do Tissue Culture in Two or Three Dimensions? That Is the Question.

Robert M. Hoffman

AntiCancer, Inc., San Diego, California, USA and Laboratory of Cancer Biology, Department of Pediatrics University of California at San Diego, School of Medicine, La Jolla, California, USA

***STEM CELLS* 1993;11:105-111**

AlphaMed Press

4100 South Kettering Boulevard
Dayton, OH 45439-2092 USA
☎+513 293-8508
Fax: +513 293-7652

Concise Review

To Do Tissue Culture in Two or Three Dimensions? That Is the Question.

Robert M. Hoffman

AntiCancer, Inc., San Diego, California, USA and Laboratory of Cancer Biology, Department of Pediatrics, University of California at San Diego, School of Medicine, La Jolla, California, USA

Key Words. Histoculture • Sponge-gel matrix • Monolayer culture • Cell-cell interactions • Cell shape • Tissue architecture • Drug resistance • Gene expression

Abstract. ***Alexis Carrel*** **introduced the in vitro culture of tissues in the beginning of the century utilizing a culture system that allowed the three-dimensional growth of tissues.** ***Leighton*** **improved upon this system by developing a substrate of sponge matrices. Other methods of three-dimensional culture include collagen gels and what are known as organ culture systems on filters or meshes. In addition, cell suspensions can be converted into multicellular spheroids, another form of three-dimensional culture. Comparison of the three-dimensional culture methods with two-dimensional culture methods has shown critical differences in the behavior of biological systems in culture. For example, in vivo-like drug responses are observed in three-dimensional but frequently not in two-dimensional cultures, indicating that drug response may be a function of tissue architecture. The in vivo mechanism of drug resistance may involve alterations in cell-cell interaction which may occur in three-dimensional culture as opposed to monolayer culture. Practical applications of three-dimensional culture include the development of a drug-response assay that correlates not only with drug resistance but also with drug sensitivity and survival of cancer patients. It has been shown that gene expression may be more in vivo-like in three-dimensional cultures than in two-dimensional monolayer cultures. For example, tumor antigens may be expressed in three-dimensional culture and not in monolayer culture. Thus, future studies utilizing three-dimensional cultures may significantly enhance our understanding of gene expression and resistance to drugs and enhance the efficacy of cancer chemotherapy by correctly predicting active drug regimens for individual patients.**

Correspondence: Dr. Robert M. Hoffman, AntiCancer, Inc., 5325 Metro Street, San Diego, CA 92110, USA.

Received December 11, 1992; accepted for publication December 11, 1992. 1066-5099/93/$5.00/0

Introduction: The Origins of Three-dimensional Culture

Alexis Carrel, in the early part of the century, was able to culture a small fragment of the heart of an 18-day-old chick embryo after explantation on hypotonic plasma [1]. The heart tissue continued to be cultured over a period of three months, during which it was passaged 18 times while remaining viable and, most importantly, continuing its rhythmic beating. Carrel's result was the first demonstration that tissues in vitro could retain normal function over a long period of time. Carrel understood at that time that it was difficult to induce a cultured tissue to increase in size and postulated that the proliferation rate of the cells was limited by accessibility of the cells to the nutrient medium. He observed that the peripheral cells grew most readily and that the internal cells became necrotic due to lack of access to nutrients. To overcome the lack of access of nutrients to the internal cells of the culture, Carrel began to culture the tissue on a silk veil which prevented the plasma of the culture medium from retracting and becoming spherical which would limit the access of the internal cells to the medium. Therefore, in 1912, Carrel set down the tenets of three-dimensional culture—or "histoculture" as it is now known [2].

In the early 1950s, *Leighton* [3-10] improved on Carrel's idea of three-dimensional culture. Leighton's major contribution was the introduction of the idea of a sponge matrix as a substrate for the culture of tissues. Leighton's original experiments were on cellulose sponges which were surrounded by plasma clots contained within glass tubes. He placed one to four fragments of tissue, ranging in size from 1 to 5 mm^3 on the sponges,

along with a single drop of chicken plasma and a drop of diluted chick embryo extract. After the plasma clotted, the tissue fragment firmly adhered to the sponge, and Leighton then added the nutrient mixture and placed the culture in a roller apparatus to stimulate nutrient accessibility to the tissues. Leighton also used natural and gelatin sponges for their ability to support the organized aggregations of cells and found that collagen-treated cellulose sponges were optimal for supporting the maintenance and growth of native tissue architecture. Different tissue types were grown by Leighton on the sponge-matrix cultures. Leighton made a number of important observations; for example, when C3HBA mouse-mammary adenocarcinoma cells were grown on sponge-matrix histoculture, he found that the cells aggregated in a manner similar to that in the original tumor. Distinct structures were formed within the tumors such as lumina and stromal elements, with some of the glandular structures similar to the original tumor. Leighton was also able to grow in the sponge-matrix cultures normal tissues such as chick-embryo liver, and he observed that the epithelial cells proliferated and formed glandular structures. On the other hand, when Leighton cultured hepatoma cells in sponge-matrix culture, they behaved differently from the normal liver cells and grew in a loosely-packed arrangement as opposed to normal liver cells. It is important to note that, although stromal tissue is present and functional in sponge-gel histoculture, the fibroblast cells are relatively quiescent and do not dominate these cultures. Leighton has since taken three-dimensional sponge-gel histoculture to a higher level in what he calls histophysiologic gradient culture [11]. This type of culture is carried out in chambers such that the metabolic exchange between the pool of medium and the culture chamber takes place by diffusion across a collagen membrane roof of the chamber, thereby mimicking diffusion in tissues. Very realistic tissue formation takes place in such gradient cultures.

Another approach to three-dimensional culture is to embed cells or tissue fragments in collagenous gels. For example, *Nandi* and colleagues have utilized collagen prepared from rat tail to embed cells or tissues. When clusters of cells were used, Nandi and colleagues [12] observed the formation of duct structures that radiated into the matrix of the gel in three dimensions. Nandi and colleagues observed that the cells grew for a period of over several weeks, with proliferation occurring mainly at the edge of the cell mass, and they observed that very few fibroblasts grew in the system. When mammary tissue was cultured in the collagen gels, it resembled histologically the tissue in vivo. It is important to note that Nandi and colleagues observed that if the cells were plated in monolayer culture, unlike the collagen gels, there was a decrease in cell number as a function of time. Nandi et al. speculated that an important reason for successful growth of mammary tissue-like structures in collagen gels, as opposed to monolayer culture, is that the shape of the mammary cells differed in the collagen gels in three dimensions as compared with monolayer culture. Many of the advantages of sponge-matrix culture described by Leighton may also be attributed to allowing the cells to maintain their normal shape.

Other approaches to three-dimensional culture include what is known as "organ culture" or "culture on filter or mesh supports." Among the initial developers of this strategy in the earlier part of the century were *Strangeways* [13] and *Fell* and *Robinson* [14]. Originally, tissue explants were grown on lens paper in a watch glass which contained liquid culture medium, but more recently this approach has utilized filters or mesh. Authors such as *Browning* and *Trier* [15] found that it was critical for some tissues to keep the cultures at the air-liquid interface. Methods for preventing total immersion, include attachment of a fragment of tissue to a matrix or platform that raises it above the medium level or a rocking culture chamber that alternatively exposes the culture to the medium and the atmosphere.

Another approach to three-dimensional culture is the use of "proto-tissues," which are aggregates of cells which form "spheroids." This approach was developed 20 years ago by *Sutherland* and colleagues [16] who formed aggregates of individual cells into a spherical structure by rotating the cells in spinner flasks.

Critical Comparisons Between Three-dimensional Culture and Two-dimensional Monolayer Culture

There is a substantial amount of evidence that cells growing in three-dimensional culture are more resistant to cytotoxic agents than cells in monolayer culture or dispersed culture. For example, *Bhuyan* compared cells in spheroids versus

single-cell suspensions and found that spheroids were more resistant to cytosine arabinoside and methotrexate by greater than 11-fold and greater than 125-fold, respectively [17]. Bhuyan and colleagues have also shown that monolayer cultures of colon carcinoma cells are sensitive to Piercidin C in contrast to colon tumors in vivo and, importantly, three-dimensional slices of tumors grown in vitro which are resistant to the drug [18]. Many other studies have demonstrated elevated levels of drug resistance of spheroids compared with cell monolayers [19].

Initially, investigators attributed drug resistance of spheroids to poor diffusion of the drugs to interior cells of the structures and the relatively high proportion of the resting cells in spheroids, which could be a result of nutrient deprivation and hypoxia. However, *Heppner* and coworkers performed experiments that demonstrated that three-dimensional structure itself accounts for drug resistance rather than just simple inaccessibility to nutrients [20-22]. Heppner and colleagues embedded cell clusters or fragments in collagen gels, exposed the culture to various cytotoxic drugs and compared the drug responses of the same cells in monolayers. They found that the increased resistance in the three-dimensional cultures could be up to 1,000-fold greater than in monolayer cultures, for example, with melphalan. In three-dimensional histocultures in collagen, the cells grew even in the presence of drug concentrations that reduced survival in monolayers to less than 0.1% of controls. Heppner and colleagues observed that if the cells from the collagen were replated as monolayers they became sensitive again. The key experiment done by Heppner and colleagues which demonstrated that the tissue architecture itself is critical in determining sensitivity was when monolayer cultures were treated with melphalan and 5-fluorouracil and subsequently transferred to collagen gels, the cells became highly resistant to these drugs. Thus, the cells were exposed to the drugs as monolayers where the drugs can access the cells readily and diffusion is not limiting. But once the cells were transferred after drug exposure to a three-dimensional structure, the structure seemed to confer high resistance to the drugs.

In this light, an illustrative set of experiments is described comparing drug resistance in tumors grown in animals with the drug resistance of the tumors in monolayer versus three-dimensional culture. *Teicher et al.* [23] serially passaged the EMT-6 tumor in mice that were treated with cisplatin, cyclophosphamide and thiotepa over a six-month period, with a total of 10 passages. This treatment induced highly-resistant tumors in vivo. However, when these tumors were grown as monolayer cultures, they were as drug-sensitive as the parental cells. It was concluded by the authors that certain types of drug-sensitivity may only be expressed in vivo. However, when *Kerbel et al.* [44] grew these same in vivo drug-resistant tumor cell lines as spheroids in three-dimensional cultures, resistance was observed up to almost 5,000 times that of the parent with certain drugs, for example 4-hydroperoxycylophosphamide (4-HO_2-CTX), the active form of cyclophosphamide used in vitro. High resistance was also observed to cisplatin and thiotepa. This resistance was not observed in monolayer culture, even when the monolayers were cultured on laminin or matrigel. Kerbel and colleagues observed that, while the parental nonresistant tumor lines formed spheroids that were loose and grape-like multicellular aggregates that were ellipsoid in shape, every resistant tumor sub-line formed a very highly compact spheroid. It should also be noted that in vitro exposure of the parental EMT-6 cells to 4-HO_2-CTX induced the formation of compact spheroids just as the in vivo exposure did as described above. Thus, the experiments by Kerbel reconfirm that cells in a three-dimensional configuration can be much more drug resistant than monolayers of the same cells in vitro and for the first time demonstrate that three-dimensional cellular configurations may become resistant to supra-pharmacological doses of drugs by forming compact structures.

It should be noted that Leighton, in the early 1950s, observed that tumors grew as aggregates in histoculture and suggested that these aggregates were the units of metastasis rather than single cells by observing their migration in the sponge-gel matrices. In this light, *Kerbel et al.* (personal communication) have observed that the EMT-6 cells selected to be highly drug resistant in vivo are highly metastatic. These results have deep implications in that, if drug resistance generated in vivo is indeed mediated by formation of tight aggregates of the tumor cells, especially as they migrate as emboli throughout the body, then the tight aggregates may promote metastasis. Thus, the generation of drug resistance could greatly enhance the malignancy of tumors. Therefore, failure of drug treatment in patients may have a double ramification: not only will the tumor still remain viable despite drug treatment, but also the tumor

could become more highly malignant because of the formation of highly aggregated emboli.

Greater resistance in three-dimensional versus two-dimensional culture extends also to radiation. *Olive* and *Durand* [24] observed that Chinese hamster V79 cells grown as spheroids in suspension culture are more resistant to killing by ionizing radiation than are the same cells grown as monolayers. They found that the rate of DNA repair to the radiation damage was faster in the DNA of cells in spheroids than in monolayers. Differential responses were found to be lost eight hours after separation of the spheroids into individual cells which corresponded to their loss of radioresistance. They also observed that spheroid cells showed fewer numbers of induced mutations per unit of radiation. Possibly DNA repair is also enhanced after chemotherapeutic-induced damage to the DNA in spheroids, or other three-dimensional configurations compared with monolayers, thereby possibly contributing to the enhanced drug resistance frequently seen in three-dimensional cultures.

The Influence of Three-dimensionality on Gene Expression and Maintenance of Specific Antigens

A variety of experiments indicate that tissues grown in three-dimensional culture are more likely to retain in vivo patterns of gene expression than are cell monolayers. In 1977, *Emmerman et al.* [25] made the observation that, in response to certain hormones, normal mouse mammary epithelial cells cultured in vitro undergo a specific differentiation-associated change, that is, secretion of the milk protein casein. Notably, this hormone-induced change occurred only when the cells were cultured on floating, detached collagen gels—not when they were cultured on attached gels or on plastic. The authors postulated that the shape of the cultured cells was important to their response to hormone. In further investigation of this phenomenon, *Bissell et al.* [26] reported that a significant fraction (30-40%) of mammary epithelial cells cultured on floating gels released β-casein after six days in culture compared with only 2-10% of control cells cultured on plastic.

A number of studies have compared the expression of tumor-specific antigens in cultured tumor cells and tissue in vivo. *Schlom et al.* [27], for example, reported that 44% of human breast carcinoma and 80% of colon carcinoma biopsy specimens tested express the tumor-associated glycoprotein TAG-72. However, only ~4% of breast cancer cell lines and ~6% of colon cancer cell lines express the antigen in monolayer culture. Moreover, in cell lines that were positive for the antigen, expression was traced to a small fraction of cells in each culture. When these workers grew the cell lines as monolayers on extracellular matrix proteins such as collagen, laminin, fibrin and fibronectin (whether in serum-containing or serum-free medium), they saw no enhancement of TAG-72 expression. In contrast, they did see a significant increase in TAG-72 expression when they grew a human colon cancer cell line in three dimensions as spheroids or in agar. Upon s.c. injection into nude mice, the monolayer-cultured cells developed into tumors that expressed TAG-72 at a level comparable to that seen in the metastatic tumor mass from patients. Studies from our laboratory have shown that, when tissues are explanted and histocultured on a collagen-gel-sponge matrix, the levels of TAG-72 as well as carcinoembryonic antigen (CEA) expression are similar to those found in the precultured material derived from the patients [28]. Similarly, *Menezes et al.* [29] have shown that cells from a parotid-derived muco-epidermoid carcinoma were negative for mucin expression when cultured as monolayers, but were positive when grown in agar in three dimensions.

Recent experiments in *R. Kerbel's* laboratory [30], have shown that MDA-MB-231 breast cancer cells in monolayer cultures constitutively produce high levels of transforming growth factor-β (TGF-β) mRNA, and these levels are not affected by the addition of exogenous TGF-β. In contrast, spheroid configurations of the same cells produce TGF-β mRNA only in response to exogenously added TGF-β. In addition, the TGF-β secreted from monolayers is inactive, whereas that secreted from spheroids is active. This and the other examples cited above illustrate that the pattern of gene expression in cultured cells can vary greatly according to their configuration and that the three-dimensional configuration may more fully approximate the in vivo situation.

Application of Three-dimensional Histoculture

Utilizing three-dimensional histoculture, we have developed a drug-response assay for human tumors that allows surgical tumor specimens to maintain their native tissue architecture and func-

tion for long periods when cultured on sponge-gel matrices [31-42]. The histoculture drug-response assay (HDRA) developed with this method initially was used with the end point of [^{3}H]thymidine incorporation measured by histological autoradiography. An 86% in vitro/in vivo correlation rate of chemoresistance was observed when the responses of human tumors in the HDRA to suprapharmacological doses of drugs were compared with the responses of the same human tumors grown s.c. in nude mice exposed to maximum-tolerated doses of drugs. The in vitro/in vivo correlation of chemosensitivity varied, however, depending on the drug [41].

Subsequently, the reduction of 3-(4,5-dimethylthiasole-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) was applied as an end point in the HDRA. The chemosensitivities of 16 human tumors were determined in vitro by the HDRA with the MTT end point and compared with their in vivo chemosensitivity when grown as xenografts in nude mice. Very importantly, the in vitro/in vivo comparison of drug efficacy resulted in a 90% overall correlation of in vitro/in vivo drug response with 90% true positives and 90% true negatives [32]. Thus, for the first time, an in vitro drug-sensitivity assay could highly correlate to in vivo drug sensitivity as well as resistance [32].

In a retrospective clinical correlative trial, it has now been observed in gastrointestinal cancer that the HDRA predicts both clinical resistance and sensitivity and, very importantly, patient survival (*Furukawa T, Kubota T, Hoffman RM*, unpublished data). In this clinical trial, of the 33 patients whose tumors showed drug resistance in the HDRA, 33 failed treatment with chemotherapy. Of the nine patients whose tumors showed drug sensitivity in HDRA, six had responses to chemotherapy, for a total predictive rate of 93% (39/42). In a study relating drug sensitivity in the HDRA to patient survival, 32 patients with stage III and IV gastric cancer without measurable lesions after surgery were treated with chemotherapy. Of the ten patients whose tumors were sensitive in HDRA and were treated with the sensitive drug(s), nine were alive at 120 weeks after surgery. Of the 22 patients whose tumors were insensitive in HDRA and were treated with the resistant drug(s) since the patients were on protocol, the 50% survival time was 49 weeks, and only six were alive at 120 weeks. Twenty-nine patients with stage III-V colorectal cancer without remaining tumor lesions were treated adjuvantly with chemotherapy. All seven patients whose tumors were sensitive to chemotherapy in the HDRA and were treated with the sensitive drug were alive at 120 weeks, while only 14 of 22 patients whose tumors were insensitive in the HDRA and were treated with the resistant drug were alive at 120 weeks.

In a correlative clinical trial of head and neck cancer, the HDRA predicted clinical response to cisplatin with an 83% predictive-positive rate (*Robbins KT, Hoffman RM*, unpublished data).

Conclusions: The Future of In Vitro Culture

This review has indicated that three-dimensional culture offers critical advantages with respect to the maintenance of tissue structure and function, including drug sensitivity and gene expression, all of which may depend on tissue architecture. The review has also demonstrated that studies in drug resistance in three-dimensional culture may yield results relevant to the in vivo situation as opposed to monolayer culture. We have termed drug resistance in three-dimensional culture "tissue resistance," which may critically depend on altered cell-cell interactions as observed by *R. Kerbel's* group (unpublished results), as opposed to "cellular resistance" in vitro, which may depend on phenomena such as overproduction of cell membrane proteins such as P-glycoprotein to pump out drugs [43]. A critical question is whether altered cell-cell interactions determine drug resistance in vivo as well as in three-dimensional spheroids in vitro. The question also remains as to the relationship of altered tissue structure such as the tight aggregates seen in spheroids that accompany drug resistance to enhanced metastatic activity, which itself may depend on aggregate formation.

Other studies reviewed here indicate that gene expression, such as membrane antigen formation and differentiated product formation, may be more in vivo-like in three-dimensional culture than in monolayer culture [27, 28].

Practical applications of three-dimensional culture include the development of clinically relevant in vitro drug-response assays that can predict drug sensitivity or resistance and patient survival, such as the HDRA described in this review.

Thus, in the coming years, our understanding of gene function and drug resistance should be enhanced by studies in three-dimensional tissue culture or histoculture. We expect practical results such as the development of individualized

targeted cancer chemotherapy utilizing the histoculture and drug testing of cancer patients' tumor tissue in vitro.

Acknowledgment

We thank *Ms. Polly Jayne Pomeroy* for excellent word processing of this manuscript and for many years of devoted service.

References

1 Carrel A. On the permanent life of tissues outside the organism. J Exp Med 1912;15:516-528.

2 Sherwin RP, Richters A, Yellin AE, Donovan AJ. Histoculture of human breast cancers. J Surg Oncol 1980;13:9-20.

3 Leighton J. A sponge matrix method for tissue culture. Formation of organized aggregates of cells in vitro. J Natl Cancer Inst 1951;12:545-561.

4 Leighton J. The growth patterns of some transplantable animal tumors in sponge matrix tissue culture. J Natl Cancer Inst 1954;15:275-293.

5 Leighton J, Justh G, Esper M, Kronenthal R. Collagen-coated cellulose sponge: three-dimensional matrix for tissue culture of Walker tumor 256. Science 1967;155:1259-1261.

6 Leighton J. Aggregate replication, a factor in the growth of cancer. Science 1959;129:466-467.

7 Leighton J. The propagation of aggregates of cancer cells: implications for therapy and a simple method of study. Cancer Chemother Rep 1960;9: 71-72.

8 Leighton J, Kalla RL, Turner JM, Fennell RH. Pathogenesis of tumor invasion. II. Aggregate replication. Cancer Res 1960;20:575-586.

9 Leighton J, Kalla RL, Kline I, Belkin M. Pathogenesis of tumor invasion. I. Interactions between normal tissues and "transformed" cells in tissue culture. Cancer Res 1959;19:23-27.

10 Leighton J, Siar JW, Mahoney MJ. Examination of invasion by manipulating stroma and parenchyma of carcinomas in vitro and in vivo. In: Brennan MJ, Simpson EL, eds. Biological Interactions in Normal and Neoplastic Growth: A Contribution to the Host-Tumor Problem. Boston: Little, Brown and Company 1962:681-702.

11 Leighton J. Structural biology of epithelial tissue in histophysiologic gradient culture. In Vitro Cell Dev Biol 1992;28A:482-492.

12 Yang J, Richards J, Bowman PD, Guzman R, Enami J, McCormick K, Hamamoto S, Pitelka D, Nandi S. Sustained growth and three-dimensional organization of primary mammary tumor epithelial cells embedded in collagen gels. Proc Natl Acad Sci USA 1979;76:3401-3405.

13 Strangeways TSP. Tissue Culture in Relation to Growth and Differentiation. Cambridge, England: W Heffer and Sons Ltd, 1924.

14 Fell HB, Robinson R. The growth, development and phosphatase activity of embryonic avian femora and end-buds cultivated in vitro. Biochem J 1929;23:767-784.

15 Browning TH, Trier JS. Organ culture of mucosal biopsies of human small intestine. 1969; 48:1423-1432.

16 Inch WR, McCredie JA, Sutherland RM. Growth of nodular carcinomas in rodents compared with multi-cell spheroids in tissue culture. Growth 1970;34:271-282.

17 Li LH, Bhuyan BK, Wallace TL. Comparison of cytotoxicity of agents on monolayer and spheroid systems. Proc Am Assoc Cancer Res 1989; 30:2435a.

18 Smith KS, Badiner GJ, Adams FG, Wilson DK, Li LH, Bhuyan BK. Modified 2-tumor (L1210, colon 38) assay to screen for solid tumor selective agents. In: Proceedings of the Symposium on Anticancer Drug Discovery and Development. Detroit: (in press).

19 Hoffman RM. Three-dimensional histoculture: origins and applications in cancer research. Cancer Cells 1991;3:86-92.

20 Lawler EM, Miller FR, Heppner GH. Significance of three-dimensional growth patterns of mammary tissues in collagen gels. In Vitro 1983; 19:600-610.

21 Miller BE, Miller FR, Heppner GH. Assessing tumor drug sensitivity by a new in vitro assay which preserves tumor heterogeneity and subpopulation interactions. J Cell Physiol 1984;3 (suppl):105-116.

22 Miller BE, Miller FR, Heppner GH. Factors affecting growth and drug sensitivity of mouse mammary tumor lines in collagen gel cultures. Cancer Res 1985;45:4200-4205.

23 Teicher BA, Herman TS, Holden SA, Wang YY, Pfeffer MR, Crawford JW. Tumor resistance to alkylating agents conferred by mechanisms operative only in vivo. Science 1990;247:1457-1461.

24 Olive PL, Durand RE. Effect of intercellular contact on DNA conformation, radiation-induced DNA

damage, and mutation in Chinese hamster V79 cells. Radiation Res 1985;101:94-101.

25 Emmerman J, Enami J, Pitelka D, Nandi S. Hormonal effects on intracellular and secreted casein in cultures of mouse mammary epithelial cells on floating collagen membranes. Proc Natl Acad Sci USA 1977;74:4466-4470.

26 Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. Proc Natl Acad Sci USA 1987;84:136-140.

27 Horan-Hand P, Colcher D, Salomon D, Ridge J, Noguchi P, Schlom J. Influence of spatial configuration of carcinoma cell populations on the expression of a tumor associated glycoprotein. Cancer Res 1985;45:833-840.

28 Guadagni F, Roselli M, Hoffman RM. Maintenance of expression of tumor antigens in three-dimensional in vitro human tumor gel supported histoculture. Anticancer Res 1991;11:543-546.

29 Menezes CA, Shikhani A, Kock W, Griffin C, Hyams V, Richtsmeier W. Properties of a parotid-derived mucoepidermoid carcinoma tissue culture. Otolaryngol Head Neck Surg 1990;103:226.

30 Theodorescu D, Sheehan C, Kerbel RS. Tumor cells grown as monolayers or multicellular spheroids indicate autoregulation of TGF-β gene expression is dependent on tissue architecture. In Vitro Cell Dev Biol (in press).

31 Freeman AE, Hoffman RM. In vivo-like growth of human tumors in vitro. Proc Natl Acad Sci USA 1986;83:2694-2698.

32 Furukawa T, Kubota T, Watanabe M, Takahara T, Yamaguchi H, Takeuchi T, Kase S, Kodaira S, Ishibiki K, Kitajima M, Hoffman RM. High in vitro-in vivo correlation of drug response using sponge-gel-supported three-dimensional histoculture and the MTT end point. Int J Cancer 1992;51:489-498.

33 Hoffman RM. In vitro sensitivity assays in cancer: a review, analysis and prognosis. J Clin Lab Anal 1991;5:133-143.

34 Hoffman RM. Three-dimensional gel-supported native-state histoculture for evaluation of tumor-specific pharmacological activity: principles, practices and possibilities. J Cell Pharmacol 1991;2: 189-201.

35 Colangelo D, Guo HY, Connors KM, Kubota T, Silvestro L, Hoffman RM. Correlation of drug response in human tumors histocultured in vitro with an image-analysis MTT end point and in vivo xenografted in nude mice. Anticancer Res 1992;12:1373-1376.

36 Hoffman RM, Monosov AZ, Connors KM, Herrera H, Price JH. A general native-state method for determination of proliferation capacity of human normal and tumor tissues in vitro. Proc Natl Acad Sci USA 1989;86:2013-2017.

37 Robbins KT, Varki NM, Storniolo AM, Hoffman H, Hoffman RM. Drug response of head and neck tumors in native-state histoculture. Archives of Otolaryngol Head and Neck Surg 1991;117:83-86.

38 Vescio RA, Redfern CH, Nelson TJ, Ugoretz S, Stern PH, Hoffman RM. In vivo-like drug responses of human tumors growing in three-dimensional, gel-supported, primary culture. Proc Natl Acad Sci USA 1987;84:5029-5033.

39 Vescio RA, Connors KM, Youngkin T, Bordin GM, Robb JA, Umbreit JN, Hoffman RM. Cancer biology for individualized cancer therapy: correlation of growth fraction index in native-state culture with tumor grade and stage. Proc Natl Acad Sci USA 1990;87:691-695.

40 Vescio RA, Connors KM, Bordin GM, Robb JA, Youngkin T, Umbreit JN, Hoffman RM. The distinction of small cell and non-small cell cancer by growth in native-state histoculture. Cancer Res 1990;50:6095-6099.

41 Vescio RA, Connors KM, Kubota T, Hoffman RM. Correlation of histology and drug response of human tumors grown in native-state three-dimensional histoculture and in nude mice. Proc Natl Acad Sci USA 1991;88:5163-5166.

42 Wallen JW, Cate RL, Kiefer DM, Riemen MW, Martinez D, Hoffman RM, Donahoe PK, Von Hoff DD, Pepinsky B, Oliff A. Minimal antiproliferative effect of recombinant mullerian inhibiting substance on gynecological tumor cell lines and tumor explants. Cancer Res 1989;49: 2005-2011.

43 Kawai K, Kamatani N, Georges E, Ling V. Identification of a membrane glycoprotein overexpressed in murine lymphoma sublines resistant to cis-diamine dichloroplatinum (II). J Biol Chem 1990;265:13,137-13,142.

44 Kobayashi H, Man S, Graham CH, Kapitain SJ, Teicher BA, Kerbel RS. Acquired multicellular-mediated resistance to alkylating agents in cancer. Proc Natl Acad Sci USA (in press).