STEM CELLS®
Original Article

Complex Extracellular Matrices Promote Tissue-Specific Stem Cell Differentiation

DEBORAH PHILP,[a] SILVIA S. CHEN,[b] WENDY FITZGERALD,[b] JAN ORENSTEIN,[c] LEONID MARGOLIS,[b,d] HYNDA K. KLEINMAN[a]

[a]Cell Biology Section, National Institute of Dental and Craniofacial Research, Bethesda, Maryland; [b]NASA/NIH Center for Three Dimensional Tissue Culture, National Institute of Child Health and Human Development, Bethesda, Maryland; [c]George Washington University Medical Center, Washington, DC; [d]Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, Bethesda, Maryland, USA

Key Words. Matrigel • Basement membrane • Extracellular matrix • Gland formation • Chondrogenesis

ABSTRACT

Most cells in tissues contact an extracellular matrix on at least one surface. These complex mixtures of interacting proteins provide structural support and biological signals that regulate cell differentiation and may be important for stem cell differentiation. In this study, we have grown a rhesus monkey embryonic stem cell line in the presence of various extracellular matrix components in monolayer, in a NASA-developed rotating wall vessel bioreactor in vitro, and subcutaneously in vivo. We find that individual components of the extracellular matrix, such as laminin-1 or collagen I, do not influence the growth or morphology of the cells. In contrast, a basement membrane extract, Matrigel, containing multiple extracellular matrix components, induces the cells within 4 days to form immature glandular- and tubular-like structures, many of which contain a lumen with polarized epithelium and microvilli. Such structures were seen in vitro when the cells were grown in the bioreactor and when the cells were injected into mice. These tubular- and glandular-like structures were polarized epithelia based on immunostaining for laminin and cytokeratin. The cell aggregates and tumors also contained additional mixed populations of cells, including mesenchymal cells and neuronal cells, based on immunostaining with vimentin and neuronal markers. An extract of cartilage, containing multiple cartilage matrix components, promoted chondrogenesis in vivo where alcian blue–stained cartilage nodules could be observed. Some of these nodules stained with von Kossa, indicating that they had formed calcified cartilage. We conclude that extracellular matrices can promote the differentiation of embryonic stem cells into differentiated cells and structures that are similar to the tissue from which the matrix is derived. Such preprogramming of cell differentiation with extracellular matrices may be useful in targeting stem cells to repair specific damaged organs.
STEM CELLS *2005;23:288–296*

INTRODUCTION

The extracellular matrices in tissues are likely to be the first molecular components with which stem cells interact. Stem cell niches are thought to be important for programming their differentiation. These complex extracellular matrices function in tissues to provide support and store growth factors and cytokines as well as biological signals to promote and maintain cell differentiation [1, 2]. The effect of individual matrix components as well as complex matrices on differentiation of various cells has been well documented using in vitro assays,

Correspondence: Leonid Margolis, Ph.D., NIH, NICHD, Building 10, Room 10D58, Bethesda, MD 20892 USA. Telephone: 301-594-2476; Fax: 301-480-0857; e-mail: margolis@helix.nih.gov Received December 11, 2002; accepted for publication September 14, 2004. 1066-5099/2005/$12.00/0 doi: 10.1634/stemcells.2002-0109

but their effect on stem cell differentiation is less well studied. The basement membrane matrix, which underlies epithelial and endothelial cells and contacts smooth muscle, fat, and peripheral nerve cells, is highly enriched in growth factors [2] and has been shown to promote the differentiation and formation of tissue-like structures of many cell types. In particular, endothelial cells attached to a complex mixture of basement membrane components, termed Matrigel or EHS gel [3], form capillary-like structures with a lumen [4]. Likewise, freshly isolated Sertoli cells form chord-like structures, and salivary gland cells form acinar-like structures on Matrigel [5, 6]. The cells on basement membrane matrices are more differentiated based on the activation of cell type–specific genes [7–9]. For example, breast epithelial cells form glandular-like structures and produce casein when cultured on basement membrane matrix [10]. In addition, salivary gland cells produce amylase when cultured on basement membrane matrix but not when cultured on either collagen I or laminin-1, biologically active components of skin and the basement membrane, respectively [11].

Although much has been studied with basement membrane and its components, less has been accomplished with other extracellular matrices. It would be expected that such matrices also would have cell type–specific effects on cell differentiation. Recently, we developed a new matrix, an extract of cartilage, termed Cartrigel, using the same methodology with minor modifications as was used for the basement membrane [12]. In preliminary studies, this material was found to sustain chondrocyte differentiation.

In this study, we have examined the biological effects of basement membrane (Matrigel) and of cartilage (Cartrigel) extracts on the differentiation of pluripotent rhesus monkey embryonic stem (ES) cells in conventional monolayer culture and in a rotating wall vessel (RWV) bioreactor, which was shown to facilitate differentiation of various cells. In this NASA-designed device, cells grow in suspension, form large aggregates, and are subjected to minimal shear forces [13]. We find that Matrigel can promote rapid and specialized cell differentiation with more differentiated cells in the RWV bioreactor than in monolayer cultures, whereas some of the individual components of Matrigel cannot stimulate differentiation in either culture condition. Basement membrane extracellular matrix (Matrigel) promoted immature glandular/epithelial differentiation, whereas cartilage extract (Cartrigel) promoted the formation of mature and calcified cartilage.

Materials and Methods

Reagents

Polyclonal antibodies to laminin and monoclonal antibodies to class III tubulin β were obtained from Chemicon (Temecula, CA). Monoclonal antibodies to vementin (V9) were obtained from Vector laboratories (Burlingame, CA), and monoclonal antibodies to cytokeratin (AE1/AE3) were obtained from DAKO (Carpenteria, CA). Basement membrane Matrigel at 11–13 mg/ml, collagen I at 3 mg/ml, and laminin-1 at 1 mg/ml were obtained from Collaborative Biomedicals, Inc. (Bedford, MA). Using essentially the same protocol as for Matrigel preparation [3], Cartrigel was prepared from calf knee cartilage obtained from a slaughterhouse. Cartilage was cut into small pieces, frozen in liquid nitrogen, and pulverized with a mortar and pestle. The fragments were washed in high salt and extracted overnight with 2 M urea, as has been done for Matrigel preparation or with 2 M guanidine. The final Cartrigel preparation was dialyzed against Dulbecco's modified Eagle's medium (DMEM), aliquotted, and stored at –20°C.

Cell Culture

A cloned rhesus monkey ES cell line (R366.4) derived from blastocysts was obtained from WiCell Institute (Madison, WI) [13]. ES cells were cocultured with mitomycin C–treated (0.8 mg/ml for 2 hours) (Sigma, St. Louis) murine embryonic fibroblasts (MEFs) (Cell Essentials, Inc., Boston) in gelatin-coated six-well plates (Nalge Nunc, Inc., Naperville, IL) to prevent spontaneous differentiation. These cultures were grown in ES culture medium that contained knockout DMEM supplemented with 20% defined fetal bovine serum (Hyclone, Logan, UT), 1% nonessential amino acids (Invitrogen, Rockville, MD), 1 mM L-glutamine (Invitrogen), and 0.1 mM β-mercaptoethanol (Invitrogen). ES cells were split by releasing them from the culture plate with 0.8 mg/ml collagenase IV (Invitrogen) and seeded onto a new mitomycin C–treated MEF feeder layer in a gelatin-coated six-well plate.

Extracellular Matrix Studies

ES cells were released from the six-well plate with collagenase IV (0.8 mg/ml) and suspended in knockout DMEM growth medium. To determine the effect of various extracellular matrices on ES cell differentiation, Matrigel, laminin-1, collagen I, and two types of Cartrigel were added to stem cell cultures. This was done by adding each matrix in triplicate to 24-well ES cell cultures either in solution or as a substrate. In solution, these extracellular matrices were diluted in ES cell culture media. Laminin-1 and collagen I were used at concentrations of 1 and 0.5 μg/ml. Cultures were observed and photographed. Similarly, Matrigel was mixed with growth media and added to triplicate 24-well stem cell cultures at 2.0, 1.0, 0.5, and 0.25 mg/ml. Cartrigel (urea extract) was used at concentration of 5.6 mg/ml. When used as a substrate, the bottom of the 24-well plate

was coated with either 11–13 mg/ml Matrigel or 5.6 mg/ml Cartrigel. Cell differentiation was assessed by two independent observers. Each experiment was repeated at least three times in triplicate.

Bioreactor Cultures

Cultures were grown in 10 ml of growth media in a disposable RWV cell culture system (Synthecon, Houston). In control cultures, ES cells were cultured in ES cell growth medium alone. In addition, ES cells were cultured with 0.25 mg/ml Matrigel or 5.6 mg/ml Cartrigel (urea extract) in solution. ES cells from two wells of the original six-well plate were used in each of the RWV cultures. The cells were cultured for up to 2 weeks, and differentiation was assessed by two independent observers. Each condition was tested at least three times.

Tumor Growth In Vivo

Cells from the monolayer cultures and the bioreactors after 11 days and 14 days of in vitro culture, respectively, were injected into athymic nude mice with the indicated matrix components. Injections were done intramuscularly in the rear flanks or subcutaneously in the upper dorsal area. For controls, matrices alone were injected in a similar manner. Tumors were harvested from the animals 15 weeks after injection. Each experiment was repeated twice with four mice per condition. Fifty percent of the Cartrigel-injected mice formed visible rapidly growing tumors, whereas fewer tumors (one of four) were observed with the Matrigel-injected mice, but viable tissue was found in the muscle.

Immunohistochemistry and Electron Microscopy

Cells cultured in vitro in monolayer, in a RWV, and grown in vivo were fixed at various times in 0.2 M phosphate-buffered saline, pH 7.4, containing 4% formaldehyde. The tissue was sectioned and stained with either hematoxylin and eosin (H&E), von Kossa stain, or alcian blue. Immunostaining for laminin was performed at an antibody dilution of 1:500, for class III β tubulin at an antibody dilution of 1:100, for antibody to vimentin at a dilution of 1:50, and for antibody to cytokeratin at a dilution of 1:50. For electron microscopy, tissues were fixed in 2% gluteraldehyde in phosphate-buffered saline (PBS) and processed. At least two different cultures and two different tumors were examined for each antibody tested.

Transmission Electron Microscopy

The medium was removed from the cell cultures and replaced with neutral, PBS-buffered 2.5% glutaraldehyde at room temperature. The layers of cells were scraped free from the bottom of the well with a rubber policeman, transferred to a microfuge tube using a wide-mouth pipette, and pelleted. Cell pellets were mixed with warm agar, which was then solidified by cooling at 4°C overnight. The cell blocks were postfixed in 1% osmium tetroxide, block-stained in uranyl acetate, processed through graded ethanol and propylene oxide, and embedded in Spurr's epoxy. One-micron, semithin, plastic sections were cut with glass knives and stained with methylene blue, azure II, and basic fuchsin for plastic section light microscopy. Selected blocks were thin-sectioned, stained with uranyl acetate and lead citrate, and examined on a LEO EM10 electron microscope operating at 60 kV.

Results

We first examined the effects of various extracellular matrices on the differentiation of rhesus monkey ES cells in vitro (Table 1). Based on analysis of the morphology, the basement membrane component laminin-1 and collagen I had little effect on cell survival or differentiation when added as a substrate in place of the feeder layer or as a soluble reagent in the presence of the feeder layer (Table 1). The basement membrane extract Matrigel, either in solution or as a substrate in the absence of the feeder layer, induced cell growth and differentiation. By 4 days in culture, several different cellular morphologies were observed in the same well. In addition to isolated single cells, large tubular-like structures were observed along with areas of cell aggregates (Fig. 1B), whereas the control (no Matrigel or Cartrigel) cells alone formed a monolayer (Fig. 1A). By day

Table 1. Effect of various extracellular matrix components on the differentiation of monkey embryonic stem cells in vitro and growth in vivo

Matrix	Monolayer	Bioreactor	In vivo
None	Undifferentiated	Undifferentiated	None detected
Collagen I	Undifferentiated	Not done	Not done
Laminin-1	Undifferentiated	Undifferentiated	Not done
Matrigel	Immature glands	Numerous immature glands	None detected
Cartrigel	Undifferentiated	Cartilage nodules	Cartilage nodules

The cells tested in vivo were obtained from the monolayer and bioreactor cultures.

11 in culture on Matrigel, the cells had continued to form large organ-like tubular structures as well as highly condensed areas in which a clear morphology could not be determined (Figs. 1C, 1D). Based on morphological comparisons with control cell cultures, it was apparent that proliferation was increased in the presence of Matrigel and that the cells were not just migrating into clumps. Individual cells and structures within the clumps could not be easily observed with light microscopy of living samples. Therefore, we fixed, sectioned, and stained cells grown on Matrigel. H&E staining revealed that many glandular-like structures could be observed with the cells grown on Matrigel. Some contained polarized columnar epithelial-like cells without a lumen (Fig. 1G), whereas others had lumens of varying sizes (Figs. 1E, 1F, 1H).

Cells were also grown in RWV bioreactors, and the effects of laminin-1 and basement membrane Matrigel were assessed. In RWV bioreactors, the three-dimensional in vivo environment may be reflected more faithfully. With laminin-1, cells formed few polarized structures (Figs. 2A–C), similar to control cultures. A small structure shown in Figures 2A–C was highly infrequent and shows poor cellular organization. In contrast, addition of soluble Matrigel into the RWV cultures clearly increased the size of the cell aggregates with time of culture (data not shown), and the number of highly organized tubular- and glandular-like structures was much greater than that observed in the control. In addition, some areas appeared to contain muscle-like cells (Fig. 3F). There were also many more tubular- and glandular-like structures in the RWV bioreactor culture (approximately 20%–40%) (Figs. 2A–C) than in the cultures from the dishes in the presence of Matrigel (approximately 10%–20%). Staining with laminin antibodies confirmed the polarized nature of the cell clusters even when the cells did not appear polarized (Fig. 2D). We immunostained the cells in the RWV and in the tumors with markers for epithelial cells (cytokeratin, AE1/AE3), neuronal cells (class III β tubulin), and mesenchymal cells (V9). The tumor-derived polarized tubular- and glandular-like cells stained with the cytokeratin antibody, which is a marker of epithelial cells (Figs. 3A, 3B). Approximately 30% of the cells, entirely in the nonglandular areas, stained with the marker for mesenchymal cells (Figs. 3E, 3F), whereas clusters of cells were positive with the neuronal markers (Figs. 3C, 3D) only in the RWV cultures, with no neuronal cells observed in the tumors. No cells were positive for the endothelial cell lineage (data not shown). Thus, the cells in the RWV and in the tumors were of mixed cell types, with the dominant structures being epithelial.

The nature of these structures was confirmed by electron microscopy, in which they were found to appear as immature glands (Fig. 4). The untreated control cells grew predominantly

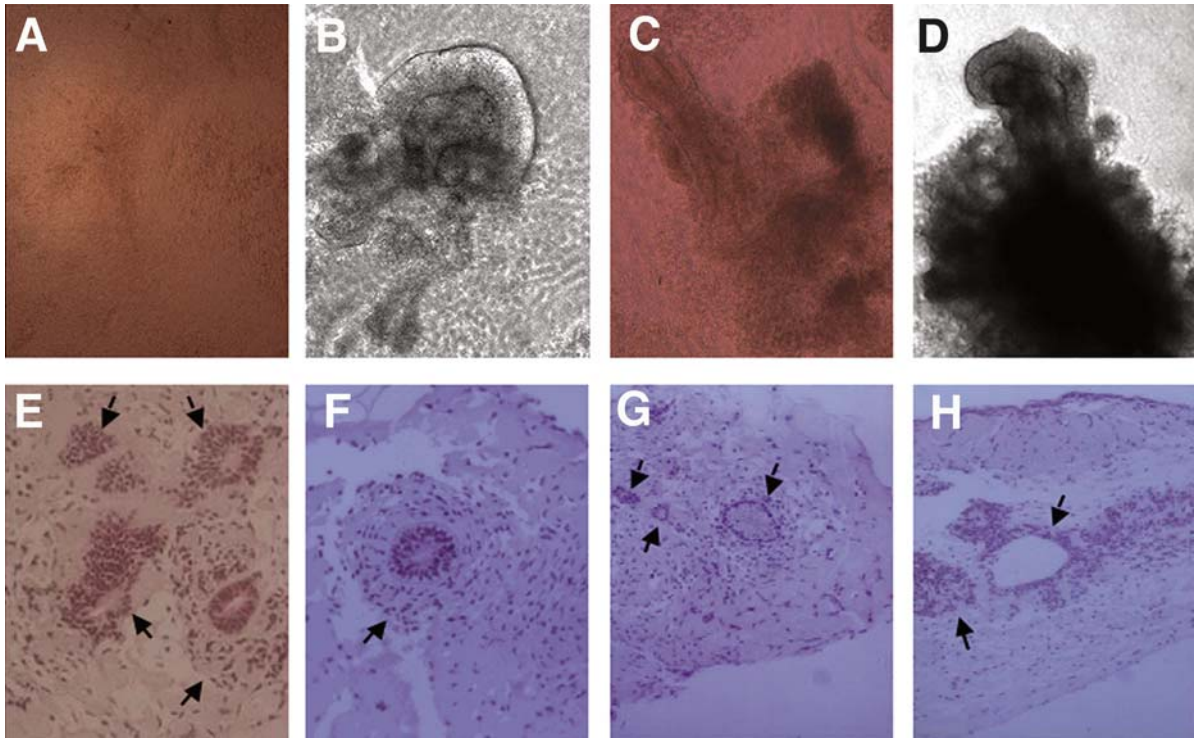

Figure 1. In vitro culture of rhesus monkey embryonic stem cells alone **(A)** and on Matrigel substrates **(B–H). (A):** Embryonic stem cells on murine embryonic fibroblasts after 11 days. **(B):** Cells on Matrigel after 4 days. **(C, D):** Cells on Matrigel after 11 days. **(E–H):** Histological section of cells stained with hematoxylin and eosin after 11 days of culture on Matrigel.

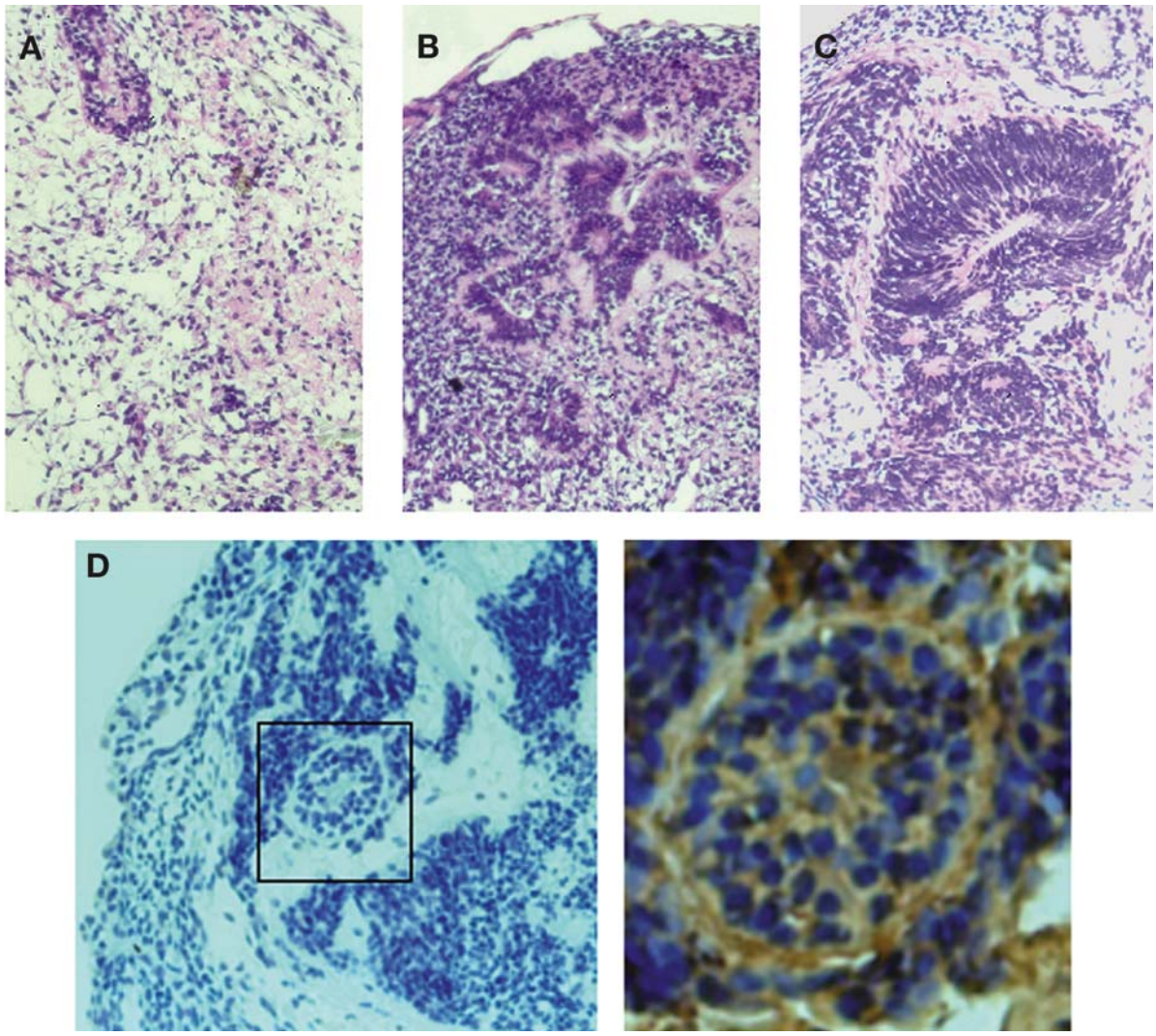

Figure 2. (A–C): RWV culture of rhesus monkey embryonic stem cells alone **(A)** and in the presence of Matrigel **(B, C)** for 30 days. **(A):** Cells alone. **(B, C):** Cells cultured with soluble Matrigel. All sections were stained with hematoxylin and eosin. **(D):** RWV culture of rhesus monkey stem cells in the presence of Matrigel and stained for laminin-1. Abbreviation: RWV, rotating wall vessel.

as flat monolayers joined to one another at their free surface by terminal bars comprised of a complex of tight and intermediate junctions and desmosomes (Figs. 4A). Pleomorphic villous processes lacking core rootlets or a glycocalyx sparsely covered the free surface. Some areas of the basal surface were coated by a basal lamina. The cytoplasm contained small amounts of intermediate filaments, consistent with vimentin, as well as thin actin filaments. Cellular debris was seen in the cytoplasm of some cells, consistent with apoptosis.

Cells grown on Matrigel mainly differed from the control cell cultures by their unique organization: aggregates of varying sizes and tubular structures. The tubular structures varied from having a single layer of cells to having multiple layers. Rare desmosomes joined lateral surfaces of the cells. Most cells were round to oval in shape, with occasional columnar cells. These cells also had terminal bars, pleomorphic microvilli, and stretches of basement membrane (Figs. 4B, 4C).

Next, we studied further differentiation of these cells in the presence and absence of laminin-1 and Matrigel upon intramuscular and subcutaneous injection. In 5 months, we did not observe in vivo growth of cells cultured in RWV or on monolayers with laminin-1. With Matrigel-injected cells, tumors did grow as previously shown [14].

In addition, we determined the biological effect of another complex extracellular matrix, Cartrigel, which was prepared from cartilage in a manner analogous to the procedure used for Matrigel. In monolayer, the cells were cultured in vitro in the presence of a Cartrigel substrate or, when it was present in solution, seemed to increase in number with some condensation, but clear structures could not be observed even after H&E histological staining (data not shown). Similarly, in the RWV bioreactor, the cell number increased several-fold, with

the aggregates of cells some 5- to 10-fold larger than controls, but no obvious differentiation was observable (data not shown). When these cells were injected subcutaneously in mice after incubation in the RWV bioreactor for 21 days, growing tumors in 50% of the mice injected were observed at the injection site within 2 months. When examined by histology, large cartilage nodules were found that stained with alcian blue (Figs. 5A, 5C, 5D). Some of the cartilage nodules appeared calcified, as demonstrated by von Kossa staining (Fig. 5B). When Cartrigel was injected alone, no tumors were observed, and the material seemed to be absorbed, because it could not be found in the tissue. This study demonstrates that complex extracellular matrices can drive cell differentiation along different pathways depending on the source of the matrix components.

Discussion

Using a pluripotent ES cell line, we demonstrate that complex extracellular matrices, like Matrigel and Cartrigel, can promote tissue-specific differentiation dependent on the type of matrix. Basement membrane Matrigel promoted epithelial/glandular-like structures, and these cells in normal

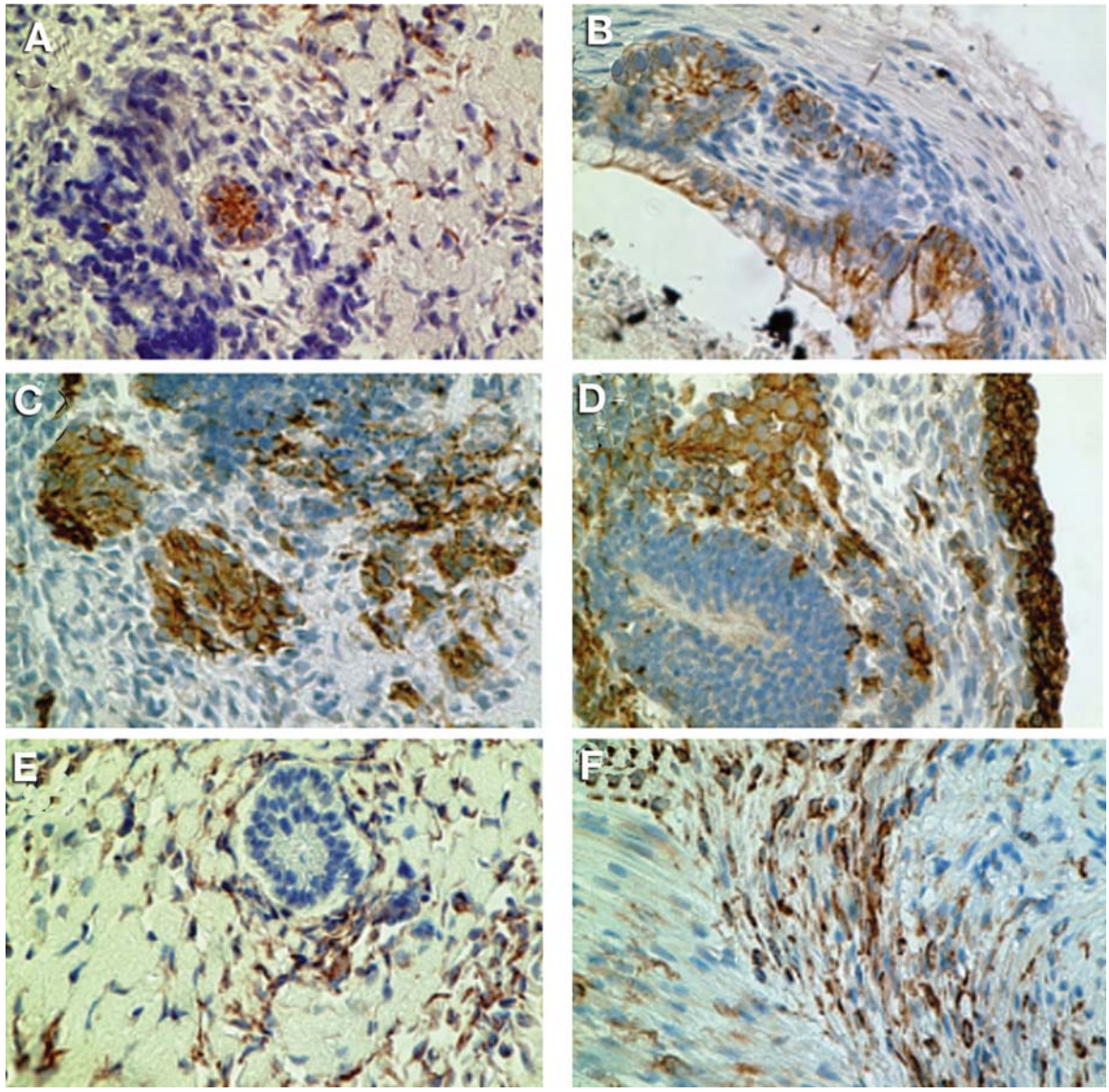

Figure 3. Immunostaining of cells grown in the RVW and in vivo in the presence of basement membrane Matrigel. **(A, B):** Immunostaining for cytokeratin in cells in RVW **(A)** and in tumors **(B). (C, D):** Immunostaining for neuronal cells in cells in RVW. **(E, F):** Immunostaining for vimentin in cells in RVW **(E)** and in tumors **(F)**. Abbreviation: RWV, rotating wall vessel.

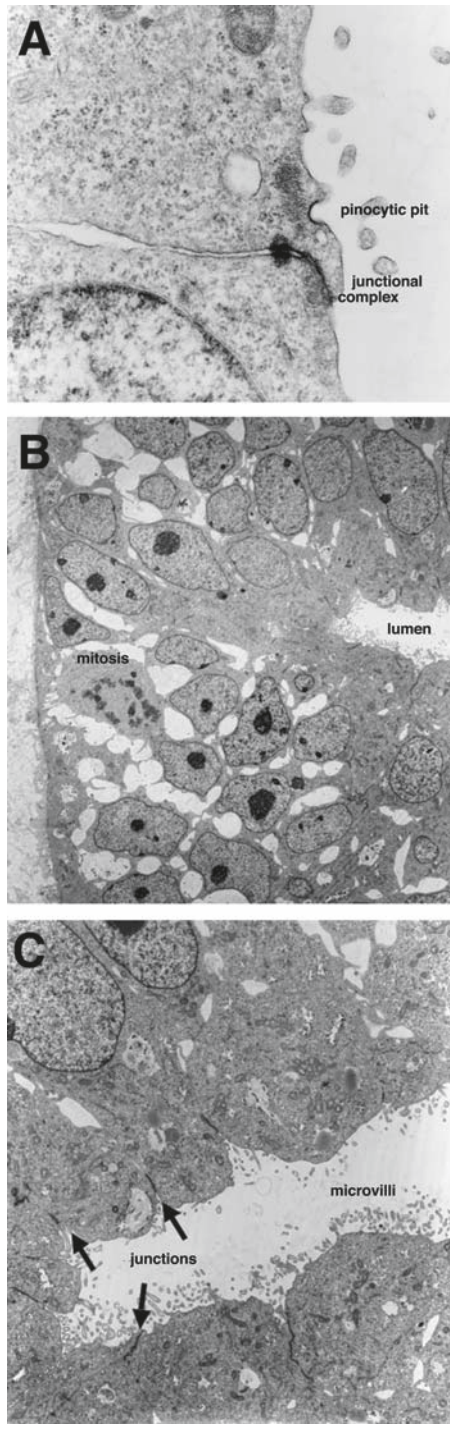

Figure 4. Electron microscopy of rhesus monkey embryonic stem cells alone **(A)** and in the presence of Matrigel **(B, C)** for 21 days in culture. **(A):** Control. The apical surface of two control cells is joined by a well-developed terminal bar. Note the presence of several microvilli and a coated pinocytotic pit. **(B):** Matrigel, low magnification. This tubular structure is comprised of several layers of cells that are partially separated by gaps. A single cell is in the process of dividing. **(C):** Matrigel, higher magnification of **(B)**. The luminal surface of the cells has pleomorphic microvilli and junctional complexes. The cells bulge into the lumen.

tissues contact a basement membrane. In addition, neuronal, mesenchymal, and muscle-like cells were observed, demonstrating the mixed cell population in the cultures and tumors. For the first time, it was shown that Cartrigel promoted chondrogenesis, with round cartilage nodules clearly visible among the undifferentiated stem cells. In this study, we used a NASA-developed RWV bioreactor, which was shown to promote aggregation of differentiated cells [15]. RWV mimics some of the aspects of microgravity and allows for study of matrices on ES cell differentiation in isotropic conditions without contacting substrates. Matrigel has been used with undifferentiated human ES cells in the past [14], but this is the first study with Cartrigel. Previously, Matrigel was found to maintain human ES cells without a feeder, but the presence of mouse fibroblast-conditioned medium was required. These cells could be expanded in culture without differentiating and did form tumors when injected into mice. Probably the difference between their finding of little differentiation with Matrigel and ours with significant differentiation was due to their use of mouse fibroblast-conditioned medium and the use of different cells. Earlier, committed stem cells from adult muscle were cultured on isolated muscle fibers with Matrigel and found to form myocytes and osteogenic and adipogenic lineages [16]. In the absence of Matrigel, these three phenotypes could not be observed unless various other factors (adipogenic cocktail, BMP-4) were added. Certainly, the additional growth factors and other components may have been important in promoting growth and reducing differentiation [17].

In other systems, exogenously added growth factors have been found to regulate stem cell growth and differentiation [18]. Various growth factors were found to specifically promote embryoid body formation and differentiation of ES cells into endoderm, mesoderm, or ectoderm with epithelial or mesenchymal cell morphologies.

With Matrigel, we saw more abundant differentiation when the cells were grown in the RWV bioreactor. This may be attributable either to the higher density of the cells that grow in aggregates or greater accessibility of nutrients. In some samples, the differentiation was more observable along the outer areas of the aggregate, suggesting that the nutrient availability was important. High cell density has been shown to be important when bone marrow stem cells, which are maintained in micromass pellets, were induced to differentiate with transforming growth factor-β3, dexamethasone, and BMP-6 [19]. Likewise, embryoid bodies were more prone to differentiate than the nonaggregated cells in the presence of Matrigel in previous studies [14]. The cell–cell contact that is more representative of in vivo tissues seems to increase cell differentiation, and this may explain the differentiation advantage observed in the RWV, which is

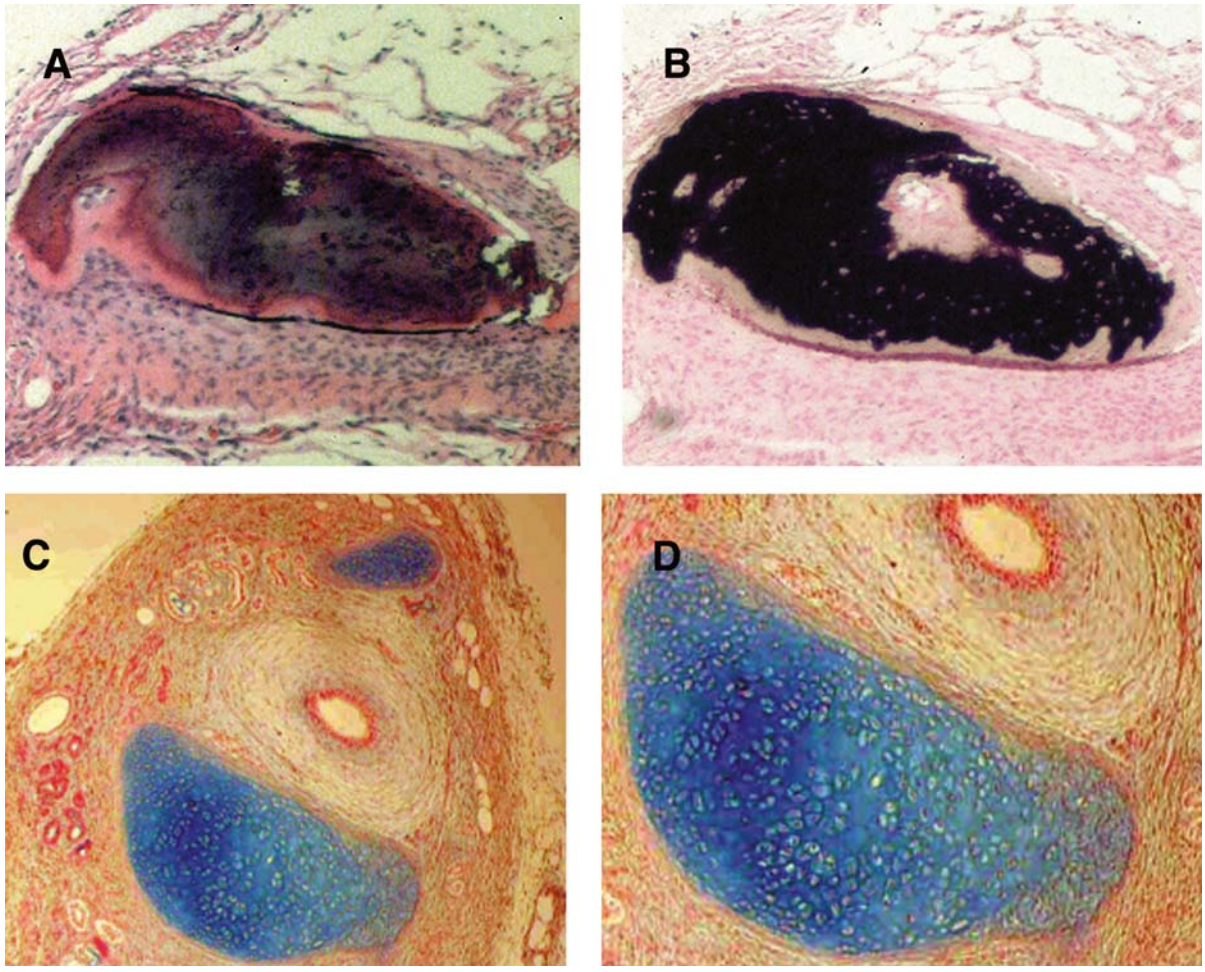

Figure 5. Histology of tumors formed after 15 weeks of injection of embryonic stem cells into mice. Embryonic stem cells were first cultured in vitro with Cartrigel and then injected. **(A):** Hematoxylin and eosin section of tumor tissue. **(B):** Von Kossa stain of tumor tissue. **(C, D):** Alcian blue stain of tumor tissue.

known to promote cell aggregation [15].

There is considerable variation in the amount and type of components present in extracellular matrices. For example, the basement membrane of the kidney glomerulus has different proportions of collagen and laminin from that of the skin basement membrane. In addition, there are different isoforms of collagens and laminins in these two tissues and different growth factors [20, 21]. Cartilage matrix contains low levels of laminin and is highly enriched in proteoglycans and collagen II [22]. It appears that each extracellular matrix contains components unique to its tissue localization. It is possible that tissue-specific matrices may further promote organ specialization.

In conclusion, we found that pluripotent cells can respond with different differentiation profiles to two distinct extracellular matrices. It is likely that tissue-specific differentiation patterns could be obtained with basement membrane isolated from specific tissues. We speculate that extracellular matrix components from other tissues, such as brain or liver, would promote tissue-specific differentiation. These new tissue extracts could be prepared in a manner analogous to what has been done with Matrigel and Cartrigel or by other methods known to extract extracellular matrix components. Being able to preprogram pluripotent stem cells before injection into patients may help alleviate the need for isolation from tissues of committed cells, which are usually limited in number. Such preprogramming may also allow for better targeting to tissues and enhanced repair or tissue replacement.

Acknowledgments

This work was supported by intramurals funds of National Institute of Dental and Craniofacial Research and National Institute of Child Health and Human Development and by the NASA-NIH Center for Tissue Culture.

References

1 Hay ED. Cell Biology of the Extracellular Matrix. New York: Plenum Press, 1991:1–468.

2 Vukicevic S, Kleinman HK, Luyten FP et al. Identification of multiple active growth factors in basement membrane matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res 1992;202:1–8.

3 Kleinman K, McGarvey ML, Hassell JR et al. Basement membrane complexes with biological activity. Biochemistry 1986;25:312–318.

4 Kubota Y, Kleinman HK, Martin GR et al. Role of laminin and basement membrane in the differentiation of human endothelial cells into capillary-like structures. J Cell Biol 1988;107:1589–1597.

5 Hadley MA, Byers SW, Suarez-Quian CA et al. Extracellular matrix regulates Sertoli cell differentiation, testicular cord formation, and germ development. J Cell Biol 1985;101:1511–1522.

6 Kibbey MC, Royce LS, Dym M et al. Glandular-like morphogenesis of a human submandibular tumor cell line by basement membrane components in vitro. Exp Cell Res 1992;198:343–357.

7 Scheutz EG, Li D, Omiencski C et al. Regulation and gene expression in adult rat hepatocytes cultured on an extracellular basement membrane matrix. J Cell Physiol 1088;134:309–323.

8 Taub M, Wang Y, Szcesney TM et al. Epidermal growth factor or transforming growth factor a is required for kidney tubulogenesis in matrigel cultures in serum-free medium. Proc Natl Acad Sci U S A 1990;87:4002–4006.

9 Vukicevic S, Luyten FP, Kleinman HK et al. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. Cell 1990;63:437–445.

10 Jones PL, Boudreau N, Myers CA et al. Tenascin-C inhibits extracellular matrix-dependent gene expression in mammary epithelial cells. J Cell Sci 1995;108:519–527.

11 Hoffman MP, Kibbey MC, Letterio JJ et al. Role of laminin-1 and TGF beta-3 in acinar differentiation of a human submandibular gland cell line (HSG). J Cell Sci 1996;109:2013–2021.

12 Elisseeff JH, Lee A, Kleinman HK et al. Biological response of chondrocytes to hydrogels. Ann N Y Acad Sci 2002;961:118–122.

13 Thompson JA, Kalishman J, Golos TG et al. Isolation of a primate embryonic stem cell line. Proc Natl Acad Sci U S A 1995;92:7844–7848.

14 Xu C, Inokuma MS, Denham J et al. Feeder-free growth of undifferentiated human embryonic stem cells. Nat Biotechnol 2001;19:971–974.

15 Jessup JM, Goodwin TJ, Spaudling G. Prospects for use of microgravity-based bioreactors to study three-dimensional host-tumor interactions in human neoplasia. J Cell Biochem 1993;51:290–300.

16 Asakura A, Komaki M, Rudnicki MA. Muscle satellite cells are multipotential stem cells that exhibit myogenic, ostogenic, and adipogenic differentiation. Differentiation 2001;68:245–253.

17 Czyz J, Wobus AM. Embryonic stem cell differentiation: the role of the extracellular factors. Differentiation 2001;68:167–174.

18 Schuldiner M, Yanaka O, Itskovitz-Eldor J et al. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells Proc Natl Acad Sci U S A 2000;97:11307–11312.

19 Seikiya I, Vuoristo JT, Larson BL et al. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chongrogenesis. Proc Natl Acad Sci U S A 2002;99:4397–4402.

20 Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn 2000;218:213–234.

21 Erickson AC, Couchman JR. Still more complexity in mammalian basement membranes. J Histochem Cytochem 2000;48:1291–1306.

22 Lee SK, Malpeli M, Cancedda R et al. Laminin chain expression by chick chondrocytes and mouse cartilaginous tissues in vivo and in vitro. Exp Cell Res 1997;236:212–222.