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Advancing Science and Technology Via 3D Culture on Basement Membrane Matrix

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Many cells in tissues are in contact with a highly specialized extracellular matrix, termed the basement membrane. Basement membranes have certain common components, including collagen IV, laminins, heparan sulfate proteoglycans, and growth factors which have a wide variety of biological activities. Extracts of basement membrane-rich tissue have yielded material suitable for studying cell–basement membrane interactions. Cells cultured in a 3D basement membrane matrix allow the in vitro modeling of cell behavior, including differentiation, apoptosis, steps in capillary formation, cancer growth, invasion, etc. It has also led to the development of widely used assays for invasion and angiogenesis and more recently for tumor cell dormancy. Importantly, stem cell culture in 3D basement membrane matrices has provided important advances that allow for expansion of these cells in feeder layer-free cultures and for studying their differentiation. 3D basement membrane culture has allowed the molecular dissection of pathways and genes important in differentiation, aided in the identification of progenitor cells, and led to the development of tissue constructs which may be models for regenerative medicine. This review will outline how this technology has led to important research assays and findings that have advanced our understanding of tissue development and disease and aided in the preclinical development of various therapeutics.

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Cell culture has been a valuable tool for studying cell behavior. Plastic substrates are two-dimensional and usually promote cellular proliferation and inhibit differentiation. The incorporation of cells into three-dimensional culture (3D culture) provides a more physiological, predictive model (Debnath et al., 2003; Yamada and Cukierman, 2007; Xu et al., 2009). In the 1950s and 1960s, collagen I gel substrates were used for 3D culture with success for culturing some, but not all cell types (Kleinman et al., 1981). By the 1970s, floating collagen I gels were used in the study of mammary epithelial cell, and subsequently, hepatocyte differentiation. In the mid 1980s, a reconstituted basement membrane matrix (termed Matrigel, BME, Engelbreth–Holm Swarm (EHS) matrix, etc.) was described. Basement membranes in most normal tissues are minute structures usually composed of insoluble proteins. In contrast, the EHS tumor produces abundant amounts of the material that can be extracted and will gel under physiological conditions. This extract was initially used as a substratum with primary isolates of Sertoli cells (Hadley et al., 1985; Kleinman et al., 1986; Kleinman and Martin, 2005) (Table 1). On this gelled substratum, Sertoli cells became highly polarized and formed columnar epithelia, and the accompanying germ cells survived and began to differentiate, whereas on either plastic or collagen I substrates, the cells grew as a flat monolayer with no germ cell survival. Moreover, when Sertoli cells were embedded within liquid basement membrane material and then warmed to 37°C, the cells formed cord-like structures with a lumen. Such structures could be transplanted back into animals wherein they remained viable, and germ cells progressed in their differentiation toward sperm. In 1986, chick spinal ganglia explants in 3D basement membrane matrix culture showed increased neurite outgrowth and myelination (Carey et al., 1986). This was the first time that myelination was observed in culture, and provided a model for studying mechanisms involved in nerve regeneration and function. Also, breast epithelial cells formed ducts, when seeded onto, or acinar-like structures when cultured within gelled basement membrane extract (Fig. 1). Interestingly, they demonstrated a large increase in casein production and endothelial cells formed capillary-like structures with a lumen containing casein (Li et al., 1987; Kubota et al., 1988). Thus, the culture of cells in 3D on basement membrane gels resulted in a more differentiated morphology for most epithelial and endothelial cell types, and elicited more physiological responses (Kleinman and Martin, 2005). While non-malignant cells remained on top of the basement membrane matrix in 3D culture, tumor cells invaded into the matrix (Kramer et al., 1986) (Fig. 1). Observations of malignant cell behavior led to the development of an invasion assay to assess tumor cell malignancy and to test various stimulators and inhibitors of invasion (Albini et al., 1987).

Growth factors are present in basement membrane matrix and the importance of these factors was demonstrated in 1990 with MDCK cells and later with osteoblastic cells (Taub et al., 1990; Vukicevic et al., 1992). These cells respond to specific factors and the removal or inactivation of some or all of the growth factors resulted in altered cell differentiation. A "growth factor-depleted basement membrane" was prepared using a high salt wash and this material is useful for studies where growth factors are added back to define their cellular roles.

Three-dimensional cultures in basement membrane gels have been used successfully for more than 20 years, with a variety of cell types and organ explants. The utility of this culture system is based on how well the cells and explants respond, and the multiple uses these in vitro systems have (Table 2). Although the cellular response is dependent on the cell type, more physiological (in vivo-like) morphology and biochemical evidence (i.e., production of specific proteins) of differentiation

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TABLE 1. Timeline of published scientific and technological advances involving 3D basement membrane culture

1985	Sertoli and germ cell survival and differentiation
1986	Isolation and characterization of basement membrane matrix; neurite outgrowth and Schwann cell myelination of spinal ganglia explants; tumor cell invasion of basement membrane
1987	Invasion assay developed; mammary epithelial cells form acini and produce casein
1988	Endothelial cell capillary-like tube formation
1990	Growth factor reduce basement membrane matrix functional study; vessel outgrowth with pericytes from aortic ring explants
1992	Sporogonic development of malaria parasite
1997	Yolk sac stem cells form capillary-like structures
2001	Basement membrane used to replace stem cell feeder layers
2002	Human embryonic stem cells characterized by formation of capillary structures
2008	Mesenchymal stem cells undergo chondrogenesis
2009	Basement membrane defined as best stem proliferative substratum (>30 passages)

TABLE 2. Advantages and disadvantages of 3D basement membrane culture

Advantages
Cell morphology/differentiation more physiological than 2D culture
Allows for models for defining regulatory pathways, genes, cells, etc., important in cell and differentiation at different stages of development
Provides assays including high throughput for testing reagents, inhibitors, stimulators, proteins, etc., of cell/tissue differentiation and cell invasion
Can replace feeder layers for stem cell propagation
Used to identify stem cell types
Differentiated structures on 3D basement membrane can be put back into animals
Transparent matrix for easy visualization
Disadvantages
Not all cell types respond to basement membrane matrix
Human basement membrane matrix not widely available
Response of cells and tissues may be limited to a certain stage
Optimal concentrations and conditions need to be defined
Not in vivo matrix for all cells

were observed. Such controlled in vitro differentiation allows for characterization and dissection of the regulatory pathways involved. Several 3D in vitro culture systems have provided assays for testing reagents (inhibitors or stimulators) of cell differentiation. For example, vascular endothelial cells form capillary-like structures with a lumen when seeded onto basement membrane gels, and this differentiation has been widely used to test regulators of angiogenesis (Benelli and Albini, 1999). These culture systems are also used to identify regulatory genes in transfection or knockdown studies (Grant et al., 1995), and they have been adapted for high throughput screening of angiogenic regulators as well.

More than 600 articles have been published involving 3D basement membrane culture and stem cells. A major breakthrough has been the ability to replace feeder layers, composed of irradiated cells, with a basement membrane substratum for the propagation of various stem cells in the undifferentiated state, or for directed differentiation (Xu et al., 2001; Philp et al., 2005; Hakala et al., 2009). The culture conditions depend on the source of the cells. Such differentiation has aided in the characterization of stem cells.

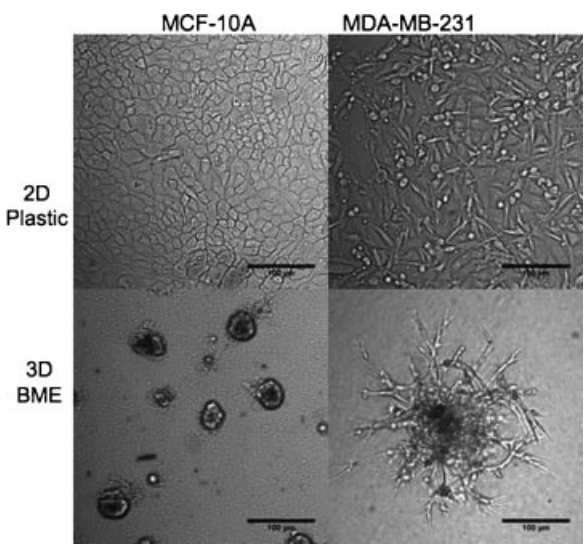

Fig. 1. Morphology on plastic versus 3D basement membrane matrix of both normal and malignant breast cells. Cells in the left two parts are normal (MCF-10A) whereas cells in the right two parts are malignant (MDA-MB-231 cells).

In some cases, these tissue- or stem cell-derived differentiated cells can be successfully implanted in animals to effect organ survival or tissue repair (Zimmermann et al., 2004). Future work in this area holds much promise.

There are a number of cautions to be taken when using 3D basement membrane culture (Table 2). In vivo, the amount and type of components in the basement membrane vary depending on the tissue type and even on the stage of tissue development. Given the small amount of basement membrane in tissues, it is not feasible at this time to provide cells or explants in 3D culture with their exact tissue matrix counterpart. It is therefore expected that not all cells will respond in 3D basement membrane culture as currently employed. Furthermore, the response of some cells may be partial differentiation/morphogenesis due to lack of an appropriate factor(s) in the matrix. Most importantly, there are various components in the basement membrane that need to be optimized depending on the cell type. For example, the type and levels of growth factors needed by cells and explants varies as described above for MDCK cells (Taub et al., 1990). In addition, the protein concentration (i.e., stiffness or pliability) of the substrate is important for cell behavior (Yamada and Cukierman, 2007). Thus, while basement membranes have many advantages for use in culture, there are some limitations and disadvantages. Clearly, some cells need to have the basement membrane substrate "customized" for optimal in vitro activity.

Basement Membranes

In vivo, basement membranes are very thin extracellular structures that underlie epithelial and endothelial cells and surround nerve, muscle, and fat cells. Many cells that do not contact a basement membrane in mature tissue still express cellular receptors and respond to this matrix in vitro. For example, chondrocytes produce "pearls" of cartilage when cultured on the surface of basement membrane gels (Bradham et al., 1995). Because basement membranes are small thin structures in vivo, initial uses in culture involved readily dissected lens capsule and amnion which are basement membrane-enriched. The EHS tumor that produces abundant basement membrane matrix has been used for decades as a source to isolate proteins, such as collagen IV, laminin, nidogen/entactin, and heparan sulfate proteoglycan. A 2 M urea extract of EHS tumor tissue yields a mixture of the major basement membrane components, which reconstitutes under physiological conditions and temperature and is biologically active (Kleinman et al., 1986). This material is available commercially under the names Matrigel, BME, and EHS matrix. It is sold in several different forms: with high protein concentrations (17–21 mg/ml), regular protein concentrations

(10–16 mg/ml), and with reduced growth factors. This tumor-derived basement membrane is a liquid at 4°C, and a gel at 24–37°C due to the interactions of the components and especially laminin self-assembly. Laminin is the most abundant component and the major gelling factor. Adding type IV collagen can increase the stability of the matrix for long-term culture. In addition to the major components listed above, growth factors such as TGF betas, EGF, FGF, PDGF, and IGF are also present. Various isoforms of these components, as well as different amounts in tissues, provides tissue-specific basement membrane matrices. Based on the amount and type of components in commercially available, tumor-derived matrix, this material is more embryonic-like in composition which may explain why so many cell types, including stem cells, and tissue explants, respond to it.

Technical Issues With Use of Basement Membrane Matrix in 3D Culture

Cell culture can be an "art" where the conditions, such as media, serum type, concentration, growth factors, etc., have to be tested to obtain optimal results. Cell biologists have struggled to maintain certain primary isolated cells in culture. While many cells adapt easily to 3D basement membrane culture, there are still some cells and explants that require definition of optimal conditions (Table 3). The actual amount of the basement membrane put into the culture dish can regulate cell morphology (Fig. 2). This was demonstrated in 1988 when researchers slightly tipped the culture dish while the basement membrane was gelling and obtained a so-called "matribeach" (Kliman and Feinberg, 1990). They found different morphologies of trophoblast cells, depending on the thickness of the gel beneath them. We now know that matrix "stiffness" or "compliance" is an important determinant of cell behavior (Johnson et al., 2007). Surprisingly, passage number after removal from liquid nitrogen can also affect cell behavior (Fig. 3). Cells apparently need to recover and fully re-adapt to the culture environment. Cell number will also have an effect on the morphology/differentiation of the cells in 3D basement membrane gels (Fig. 4A–C). Small numbers of breast cells yield small colonies, while larger numbers of cells yield interconnecting structures. With endothelial cells in 3D basement membrane culture, too few or too many cells will result in incomplete capillary-like structures or monolayers, respectively. Finally, time in 3D culture is important (Fig. 4A–C). Some cells form structures quickly (endothelial cells assemble into capillary-like structures within 6 h) while other cells types (breast cell formation of acinar structures) require more time. Thus, there are multiple variables that must be considered in 3D basement membrane culture to obtain optimal results. For many cell types, these conditions have been thoroughly defined and the information is published.

TABLE 3. Variables affecting 3D basement membrane culture

Matrix
Source
Components
Protein concentration
Thickness/stiffness/pliability
Cell
Passage number after thawing or in primary culture
Number of cells plated
Type: normal, malignant, stem, primary, and established line
Stage of differentiation
Tissue
Developmental stage
Origin/type of tissue
Time in culture
Medium
Serum amount and type
Growth factors, cytokines, hormones, minerals, vitamins, etc.

Epithelial Cells

A variety of epithelial cells have been successfully cultured on basement membrane gels (Kleinman and Martin, 2005). The responding cells generally ceased proliferation and developed a polarized morphology. Like breast cells (Fig. 1), other acinar cells from pancreas, salivary gland (Fig. 5), prostate, oviduct, etc., form acinar structures. Other cell types formed more specialized structures when seeded onto basement membrane matrix. For example, primary bone cells and an osteoblast cell line (MC3T3-E1) form long interconnecting cell processes similar to the canalicular network in bone (Vukicevic et al., 1990). Specialized proteins are produced by the differentiated cells that are not produced in 2D culture. For example, hepatocytes produce albumin and cytochrome P450 (Schuetz et al., 1988) and the bone cells produce alkaline phosphatase and deposit mineral in 3D basement membrane culture but not in 2D culture (Vukicevic et al., 1990). Although the acinar structures formed by breast and salivary gland cells appear similar, the breast cells have increased casein production (Li et al., 1987) while the salivary gland cells produced cystatin (Hoffman et al., 1996). Clearly, epithelial cells respond with distinct phenotypes. Furthermore, a reduction in apoptosis has been reported for breast epithelial cells in 3D basement membrane culture, which promotes the survival of the cells that are in direct contact with the matrix, but apoptosis is induced in the luminal cells resulting in glandular morphology.

Isolated Endothelial Cells and Vessel Outgrowth From Explants

Endothelial cells form capillary-like structures in 3D basement membrane culture within 6 h and immortalized endothelial cells will form these structures even faster (Kubota et al., 1988). These cells are polarized with basal nuclei and they take up acetylated LDL which is characteristic of mature vessel cells. As mentioned above, this capillary-like tube formation has been used as an in vitro assay for identifying stimulators and inhibitors of angiogenesis (Benelli and Albini, 1999). The assay is in wide use, and is generally a first screen for testing angiogenic and antiangiogenic factors. For example, the "tube assay" has been used to identify an angiogenic factor in the serum of systemic vasculitis patients (Cid et al., 1993). Serum was fractionated by column chromatography and various other procedures, and the "tube assay" was used to identify the angiogenic activity in the fractions at each step of the purification. The "tube assay" is also used to identify endothelial progenitor cells (Mukai et al., 2008). Genes can also be introduced or knocked down to further define their functions (Foubert et al., 2008). The "tube assay" can be used to define endothelial genes important to the angiogenesis process. Differential DNA and tissue arrays have been able to identify molecules at specific stages (attachment, migration, tubule formation, and tubule stabilization) of the angiogenic process by harvesting the cells at various time points (Grant et al., 1995; Grove et al., 2002). It is of interest to note that one group interested in aging found that basement membrane matrix produced from tumors grown in aged animals, yield poorly differentiated capillary-like structures in 3D culture (Pili et al., 1994). They demonstrated a reduction in growth factors in the aged animal-derived matrix as a probable cause of apparently inhibited differentiation.

Various vessel explants have been used in 3D basement membrane culture with successful outgrowth of capillary-like vessels, including rat aortic ring, chick embryonic aortic arch, human temporal vessel biopsy, etc. (Auerbach et al., 2003; Lozano et al., 2008). Such assays are widely used to identify

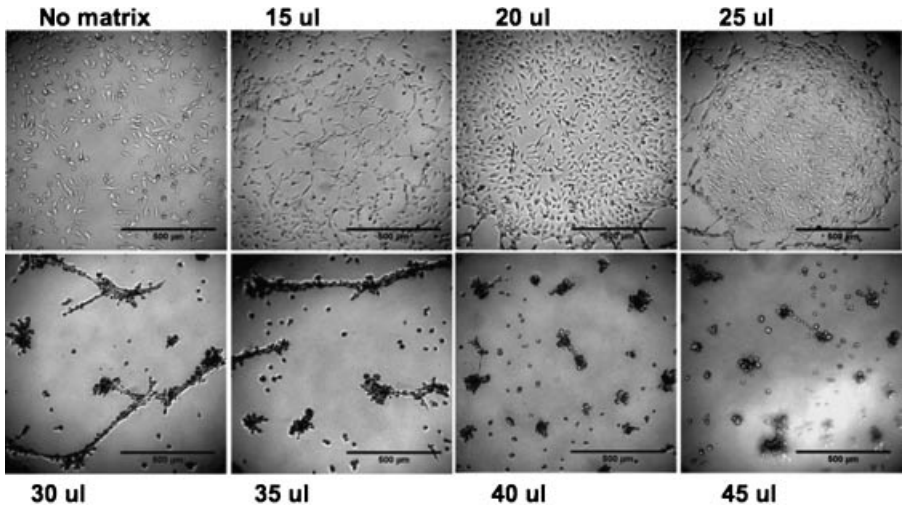

Fig. 2. Effect of thickness/gel density/stiffness on metastatic MDA-MB 231 cancer cell morphology. The assay was carried out in 96-well plates.

inhibitors and stimulators of angiogenesis and are often used as a second screen with the endothelial cell "tube assay" being the first screen. The vessel "ring" assay can also be used to test therapeutics for cancer as well as for vascular diseases. This ex vivo assay is more time consuming to set up, and depending on the age of the donor animal, sprouting can take several days. This assay measures both vessel formation and growth as opposed to the "tube assay" that measures endothelial cell migration and vessel formation. In addition, vessels from genetically modified rodents can also be assayed in this system (Nakano et al., 2007). Using such an approach with aortic rings from knockout mice, it was found that the erythropoietin receptor is important for microvessel outgrowth. These explant cultures can be further used as ex vivo assays for factors important in angiogenesis as well as for understanding vessel disease processes. Another use of this explant culture on 3D basement membrane, is to isolate the cells that grow out from the explants (Lozano et al., 2008). Human temporal artery explants in 3D basement membrane culture are a source of myointimal cells. Cells from explants of temporal vessels from patients with giant cell arteritis were found to have increased myointimal outgrowth related to disease pathology and to PDGF-AB activity. Myoinitimal cell growth could be reduced with Imatinib, an inhibitor of PDGF activity. Thus, the vessel ring assay has multiple uses for compound screening, gene discovery, and therapeutics testing.

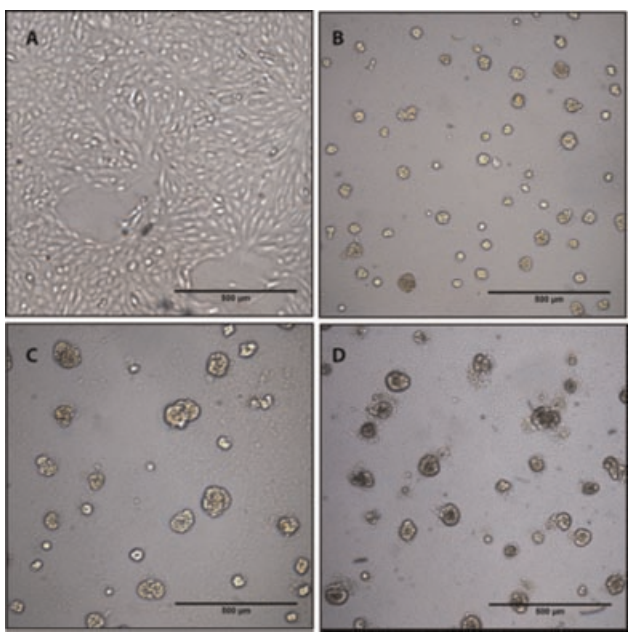

Fig. 3. Effect of passage number after thawing on breast cancer cell acinar formation. A: Subconfluent cells prior to 3D culture. B: Acinar formation at day 12 with cells from passage 1. C: Acinar formation at day 12 with cells from passage 2. D: Acinar formation at day 12 with cells from passage 3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Tissue Morphogenesis

Explanted tissues from both embryonic and adult animals have been successfully cultured in 3D basement membrane matrices and appear to proliferate and differentiate (Kleinman and Martin, 2005). Chick embryonic spinal ganglia explants were found to have increased neurite outgrowth with myelination (Carey et al., 1986). Schwann cells formed myelin sheaths around the nerve processes analogous to their morphology in vivo. Embryonic stage 12 chick neural crest tissue showed more rapid and dense outgrowth in 3D matrix than on collagen I gels (Bilozur and Hay, 1988) but the extent of differentiation was not evaluated. Furthermore, human hair growth can be studied in vitro using basement membrane matrix containing follicular dermal papilla fibroblasts and outer root sheath keratinocytes in an organotypic assay (Havlickova et al., 2004). Salivary gland rudiments from embryonic day 12 and day 13 mice which contain a few acini also grew in size and developed many more acini within 40 h (Takahashi and Nogawa, 1991; Steinberg et al., 2005) (Fig. 5). Interestingly, dissociated salivary gland cells will form new functional acinar structures when cultured in 3D basement membrane gels (Joraku et al., 2007). The life cycle of the malaria parasite can be achieved in vitro in 3D basement membrane culture (Al-Olayan et al., 2002). Thus, tissue explants can survive, grow, and in some cases differentiate in 3D

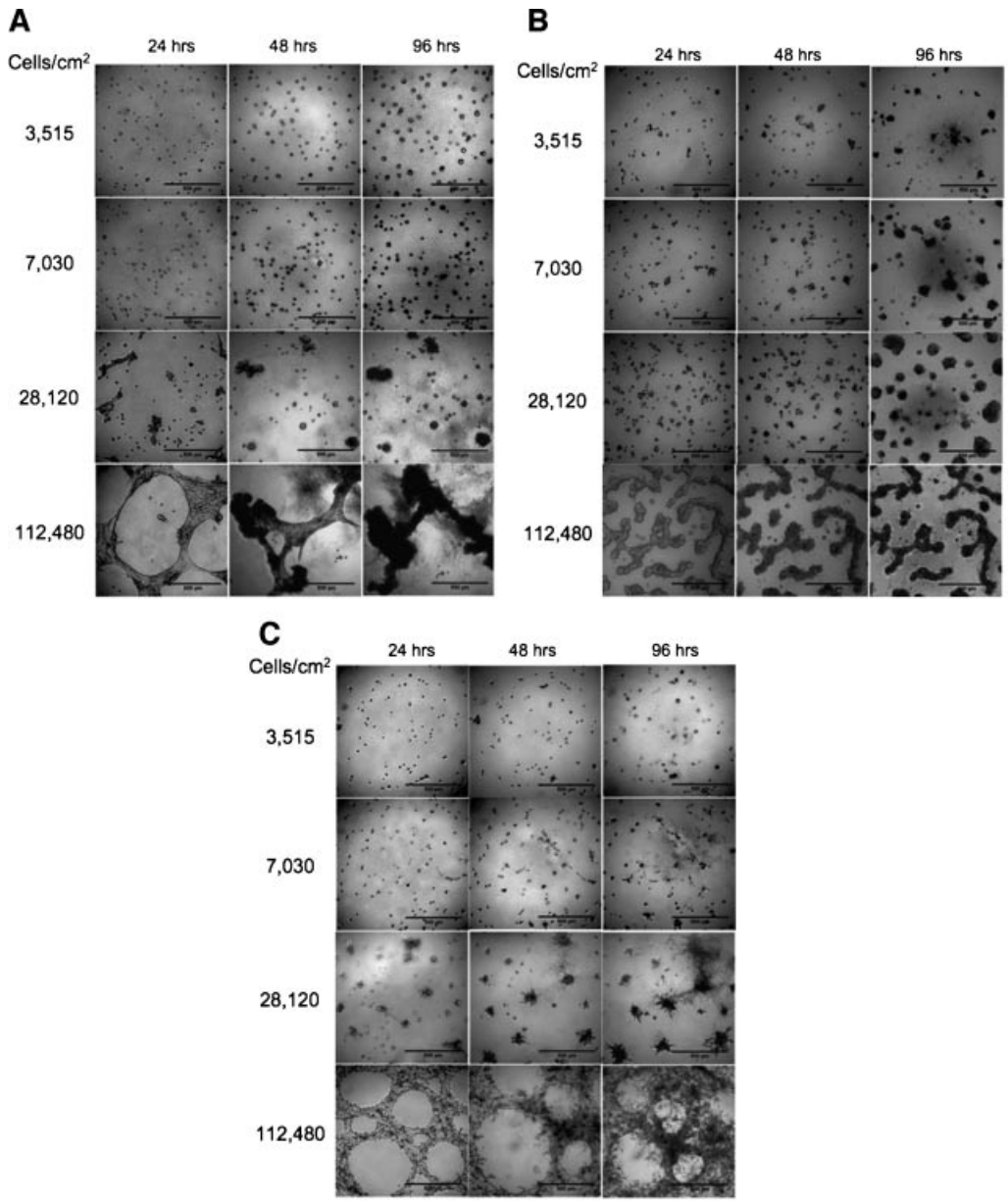

Fig. 4. Effect of cell density and time on normal, malignant, and metastatic breast cell morphology. A: MCF-10A cells. B: MCF-7 cells. C: MDA-MB-231 cells.

basement membrane culture analogous to what is observed in vivo. Thus, explants in 3D culture provide ex vivo models for studying regulators of cellular processes as well as identifying potential therapeutics for certain pathologies.

Cancer Cells

Cancer invasion and metastasis is a multi-step process. Highly malignant cancer cells are exceedingly migratory and produce many different proteases, so it is not unexpected that these cells degrade and invade the basement membrane matrix (Albini et al., 2004; Friedl and Wolf, 2008). This is apparent soon after plating when the cells extend long processes. This morphology and invasive behavior are in contrast to normal cells which differentiate. Thus, cell growth and invasion of malignant cells in 3D basement membrane culture can be used to distinguish malignant from non-malignant cells (Webber et al., 1997). In addition, tumor cells survive and proliferate well in 3D culture in basement membrane gels. Tumor growth in vivo is increased many-fold when the tumor cells are implanted with even a small amount of basement membrane matrix (Kleinman and Martin, 2005). The penetration of tumor cells through basement membrane has been widely used to measure in vitro tumor cell invasion. In these studies, the basement membrane extract is pipetted onto a porous filter with a chemoattractant placed below the filter (Albini and Benelli, 2007). The tumor cells are plated on the basement membrane and their invasion through the basement membrane and pores to the bottom surface of the filter occurs in as little as 4 h. The invasion process can be quantitated, and a number of variables can affect the success of the assay. These variables include the amount and thickness of the basement membrane (Fig. 6), cell type and level of

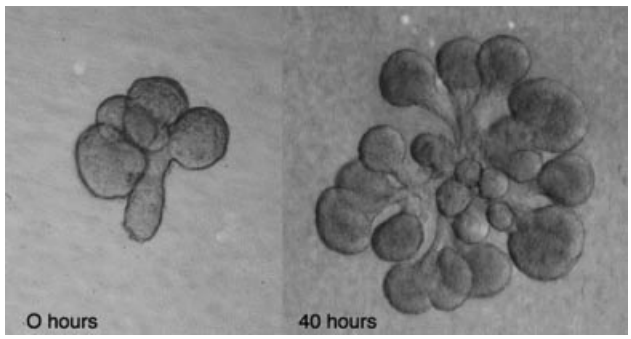

Fig. 5. Effect of basement membrane matrix on salivary gland morphogenesis. day 13 mouse embryonic salivary gland epithelium was dissected free of mesenchyme and cultured in growth factor-reduced basement membrane matrix with exogenously added FGF7 for 40 h.

malignancy, length of incubation, type and amount of chemoattractant, and cell density. The assay has been used to define malignant behavior, test various stimulators and inhibitors of invasion/malignancy, test the effects of up-regulated or knocked down genes, and define pathways important in invasion (Dong et al., 1997). The invasion assay has also been adapted for in vivo use. Needles filled with growth factors and basement membrane matrix are implanted in vivo into tumors. Cells enter the needles in a similar rate and by the same mechanisms as observed in vitro (Wyckoff et al., 2000). The in vitro assay can also be used with some non-malignant cells, including endothelial cells which degrade and invade the basement membrane during new vessel growth (angiogenesis).

The signature of genes expressed in cancers has been helpful in identifying targets for therapeutic approaches. Such an approach has now been used with normal breast tissue in 3D basement membrane culture, since normal acinar-forming cells would have genes "opposite" to those found during breast cancer development. Gene analysis of non-malignant human mammary cells that form acinar-like structures in 3D basement membrane culture has been compared with breast cancer data sets to identify down-regulated genes in the normal cells (Martin et al., 2008). This 3D signature accurately predicts breast cancer outcome and can be used as a prognostic indicator for different types of breast cancer.

Dormancy of cancer cells in vivo is a major clinical issue. Many cancers return after therapy because these cells were not susceptible to the therapeutic treatment. An in vitro assay for dormancy has been defined using known dormant cell lines and 3D basement membrane culture (Barkan et al., 2008). The dormant cells remain as small non-growing single cells and small colonies for several days. Emergence from dormancy is determined by time and various factors. This culture method allows for characterization of the pathways and genes important in dormancy maintenance and emergence, and it may have use for testing various therapeutics.

Tumor cells have also been cultured in 3D basement membrane along with endothelial cells. One mechanism of tumor dissemination proposes that tumor cells migrate along the outside of blood vessels. It has been observed in pathological specimens of melanoma that melanoma cells are often found "cuffing" the outside of blood vessels (Lugassy et al., 1999). Using already formed capillary-like structures of endothelial cells in 3D basement membrane culture, it was observed that the tumor cells attached to the matrix, migrated to the vessels, and then migrated along the vessel structures (Lugassy et al., 2002). These findings have been confirmed in explants of chick aortic rings in 3D basement membrane culture and in a shelless chick embryo assay (Lugassy and Barnhill, 2007).

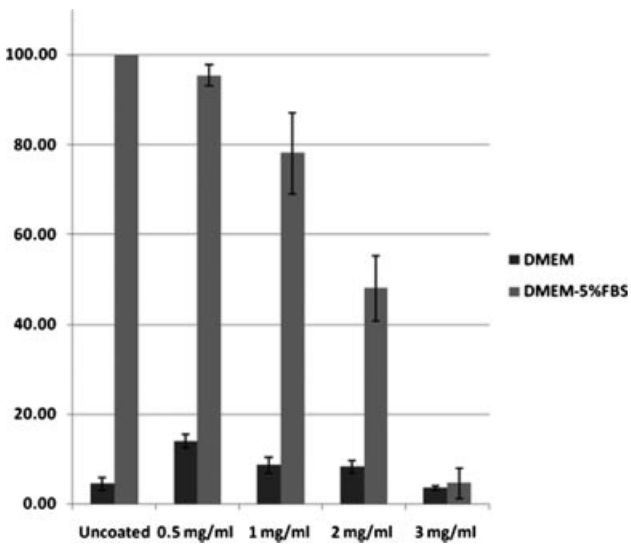

Fig. 6. Effect of basement membrane amount on MDA-MB-231 cell invasion. MDA-MB-231 cells (50,000 cells/well) were tested with varying amounts of reduced growth factor basement membrane extract on 8 μm filters with and without fetal bovine serum. The assay was carried out for 24 h at 37°C, 5% CO_2. Data are expressed as % of control.

Stem Cells

In the developing embryo, the first extracellular matrix to be synthesized is the basement membrane, which creates tissue structures and gives cells polarity (LeBleu et al., 2007). Since chick neural crest explants cultured on 3D basement membrane exhibit large outgrowth, it is not unexpected that stem cells will show multiple responses to this matrix (Bilozur and Hay, 1988) (Table 4). Isolated undifferentiated stem cells can be cultured on 3D basement membrane without the need for feeder layers (Xu et al., 2001). This has been a significant advance for this field in that the cells can be more easily cultured. Stem cells can be passaged up to 30 times on 3D basement membrane. Other extracellular matrices and artificial substrates (collagen I, fibronectin, laminin, collagen IV, sera, and various surfaces, including titanium, titanium dioxide, polylactic acid, etc.) do not support cell survival or growth beyond the first passage (Hakala et al., 2009). Stem cells are generally isolated in small numbers relative to the amount needed in vivo for tissue regeneration, so this advance in stem culture and proliferation has important implications for the feasibility of in vivo stem cell-based therapies.

TABLE 4. Scientific and technological advances with stem cells in 3D basement membrane culture

Replace feeder layers
Proliferation greater than 30 passages
Differentiation into various cell types
Characterization of cell type
Develop models for stages of stem cell differentiation
Program cells before implantation in vivo
Develop engineered tissues for implantation

Blastocyst stem cells will differentiate in 3D basement membrane culture under certain conditions to form various epithelial structures (Philp et al., 2005). In addition, culture in 3D basement membrane gels has been used to identify endothelial progenitor cells (Mukai et al., 2008). The identification of circulating endothelial progenitor cells by this simple culture method has been a technical advance for identifying cell populations for use in cell-based therapies for injured and ischemic tissues. This assay also allows for identification of and distinguishes the activities of early and late endothelial progenitor cells. Early progenitor cells formed disorganized structures while late progenitor cells formed complete capillary-like structures in 3D basement membrane culture. Interestingly, in a related assay, late endothelial progenitor cells were able to incorporate into already formed human umbilical vein capillary-like structures formed in 3D basement membrane culture while early progenitor cells caused disorganization of the pre-existing vessels. These studies have led to a better understanding of how vessels are formed in development.

Stem cells have been co-cultured with endothelial cells. For example, in acute brain slices, neural stem cells migrate on microvascular structures (Bovetti et al., 2007) and furthermore, co-culture of these stem cells with preformed endothelial cell capillary-like structures on basement membrane has been used to study stem cell migration along vascular structures (A. Puche, personal communication). Finally, co-culture of endothelial and smooth muscle progenitor cells in 3D basement membrane gels has led to increased endothelial progenitor cell survival and a stable vascular network. In related co-culture experiments, smooth muscle progenitor cells were found to cover preformed endothelial capillary-like tubes formed on 3D basement membrane (Foubert et al., 2008). If the gene Ang-1 was knocked down in the smooth muscle progenitor cells, they no longer attached to the preformed vessels, whereas exogenous recombinant Ang-1 could restore this activity. Conditioned medium from the Ang-1 knockdown smooth muscle progenitor cells also blocked tube formation by endothelial progenitor cells and this activity could also be restored by recombinant Ang-1. Co-administration of the smooth muscle and endothelial progenitor cells to an ischemia rodent model resulted in increased neovascularization but if Ang-1 was reduced in the smooth muscle progenitor cells, neovascularization did not occur confirming the importance of this gene in vivo and the utility of the in vitro assay. Culture of dissociated neonatal rat heart cells in a basement membrane/collagen I scaffold has allowed for tissue differentiation in vitro to allow for the creation of engineered heart tissue, which is then put back into the heart, where it survives for at least 8 weeks (Zimmermann et al., 2004). While the work is preliminary, it does suggest that in vitro engineered heart tissue may have application for replacement/repair of damaged or malformed heart tissue.

Summary

A number of major advances in our understanding of development and disease processes have been aided by culture on or in 3D basement membrane gels. This matrix has been used in the development of assays that are important tools for gene and drug discovery and may have additional applications in stem cell-based regenerative and palliative therapies.

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