A Novel 3D In Vitro Culture Model to Study Stromal–Epithelial Interactions in the Mammary Gland

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ABSTRACT
Stromal–epithelial interactions mediate mammary gland development and the formation and progression of breast cancer. To study these interactions in vitro, the development of defined three-dimensional (3D) models is essential. In the present study, we have successfully developed novel 3D in vitro models that allow the formation of mammary gland structures closely resembling those found in vivo. Cocultures of a human mammary epithelial cell line MCF10A and human mammary fibroblasts obtained from reduction mammoplasties embedded in either a type I collagen or a mixed Matrigel–collagen matrix were carried out for up to 6 weeks. Histological and ultrastructural analysis confirmed the formation of ductal and alveolar structures. The importance of the stromal cells was apparent in both matrices; in the collagen gels the presence of reduction mammoplasty fibroblasts accelerated the initial formation of epithelial structures, and in the mixed Matrigel–collagen gels the presence of those fibroblasts was necessary for the formation of ductal structures. These models provide an excellent system to study tissue organization, epithelial morphogenesis, and breast carcinogenesis.

INTRODUCTION
MAMMARY GLAND ORGANOGENESIS occurs throughout embryonic life, puberty, and adulthood by reciprocal interactions between the mesenchyme and epithelium.1,2 The mammary gland is a branched tubuloalveolar gland in which an epithelium lines the secretory acini and the ducts. The mesenchyme during fetal life and the stroma during adulthood contain extracellular matrix (ECM) as well as cellular components such as fibroblasts, adipocytes, immune cells, and endothelial cells. The main ECM component of the mammary gland is type I collagen, but it also contains several glycosaminoglycans, fibronectin, and basement membrane components such as type IV collagen and laminins.3–5

Surrogate animal models have contributed extensively to our understanding of how breast cancer arises and progresses.6,7 However, detailed interactions between cells and tissues are difficult to study in vivo. To reduce this complexity, epithelial cells in monolayer cultures have been commonly used to study breast cancer. However, these two-dimensional (2D) cultures resemble neither the structure nor the function of the mammary epithelium in vivo8–11 and are inadequate for assessing the role of the stroma in this tissue. The importance of the stroma as a target for mammary carcinogenesis was recently highlighted by using tissue recombination techniques.12,13 Namely, normal mammary epithelial cells can be “induced” to become tumor cells by exclusive treatment of the stroma with a carcinogen.12,13 In a complementary approach, it was also shown that normal stroma was able to induce epithelial tumor cells to form normal mammary ducts.14 Further, Kuperwasser et al. showed that organized epithelial outgrowth such as acinar and ductal

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structures could be observed only when mammary organoids were mixed with primary breast fibroblasts before engraftment into humanized mouse fat pads. In the absence of mammary fibroblasts, only nonorganized, deformed structures developed.\textsuperscript{15} These phenomena further emphasized the need to include stromal components in \textit{in vitro} studies of the mammary gland. Thus, it is important to use an \textit{in vitro} surrogate model that would mimic as closely as possible the structure and function of the human breast to study the initial steps of normal development and the carcinogenic process in this organ.

A three-dimensional (3D) model of the human breast would provide the opportunity to combine several cell types in the context of a microenvironment that resembles \textit{in vivo} conditions. Organ explants, a 3D culture developed in the 1960s and 1970s, uncovered the direct effects of specific factors such as lactogenic hormones on mammary cells while allowing for the study of tissue-level interactions.\textsuperscript{16} However, the usefulness of these cultures is limited by the short life of the explants.

Based on previous studies showing rat liver differentiation on floating collagen gels,\textsuperscript{17} Emerman and Pitelka pioneered a culture system of primary mouse mammary epithelial cells placed on floating collagen membranes in which these cells maintained morphological features such as secretory activity under the influence of lactogenic hormones throughout 4 weeks of culture.\textsuperscript{18} Later, Li \textit{et al.} introduced the use of reconstituted basement membrane (rBM), a solubilized basement membrane extracted from a mouse chondrosarcoma, as a matrix used to culture breast epithelial cells in 3D.\textsuperscript{19} This represented a significant improvement over 2D models to study the interactions among epithelial cells and between epithelial cells and basement membrane components.\textsuperscript{20,21} Three-dimensional culture systems have also been developed for other tissues such as skin, liver, lung, and endometrium using either collagen or Matrigel (BD, Biosciences, San Jose, CA), a commercially available rBM, or both as matrices.\textsuperscript{22–28} However, mammary epithelial cells embedded in rBM develop only spherical structures resembling breast acini, which are present during pregnancy and lactation, while the resting mammary gland is mainly comprised of ductal structures. As the majority of breast cancer is histologically classified as of ductal origin,\textsuperscript{29,30} it is paramount to design a 3D model in which ductal as well as alveolar structures are formed.

The aim of the current study was to develop a 3D heterotopic human breast model containing the two most prominent mammary cell types, epithelial cells and fibroblasts, within a defined matrix that would resemble that of the normal breast \textit{in vivo}. We chose MCF10A cells because they are widely used as a model for normal human mammary epithelial cells, and mammoplasty-derived fibroblasts as their stromal counterparts. Using ultrastructural and histological analyses, we observed ductal and alveolar structures closely resembling the \textit{in vivo} mammary morphology.

\section*{MATERIALS AND METHODS}

\subsection*{Chemicals and cell culture reagents}

Hydrocortisone, cholera toxin, insulin, methyl salicylate, and carmine were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), DMEM/F12, and penicillin–streptomycin solution were obtained from Gibco/Invitrogen (Carlsbad, CA). Equine serum and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT). Bovine type I collagen was purchased from Organogenesis (Canton, MA). Epidermal growth factor (EGF), Matrigel, and rat-tail type I collagen were purchased from BD Biosciences (San Jose, CA). Formalin was obtained from Fisher Scientific (Atlanta, GA).

\subsection*{Cell maintenance}

All cells used in these experiments were maintained and expanded in cell culture plastic flasks (Corning, Corning, NY). Nontumorigenic human mammary epithelial \textit{MCF10A} cells were grown in DMEM/F12 containing 5% equine serum, 20 ng/mL EGF, 0.5 \(\mu\)g/mL hydrocortisone, 0.1 \(\mu\)g/mL cholera toxin, 10 \(\mu\)g/mL insulin, and 1% penicillin–streptomycin solution. Human mammary fibroblasts obtained from reduction mammoplasties (heretofore RMF) were grown in DMEM containing 10% FBS. All cells were incubated at 37°C and 6% carbon dioxide. For coculture experiments, a combined medium (one part of \textit{MCF10A} medium and one part of \textit{RMF} medium) was used. The combined medium was previously tested in tissue culture flasks containing each cell type alone to assure proper growth and behavior of cells.

\subsection*{3D cell culture}

Type I collagen was used at a concentration of 1 mg/mL according to Paszek \textit{et al.}\textsuperscript{31} Collagen was neutralized and buffered using 1N sodium hydroxide and 10x phosphate-buffered saline according to the manufacturer's instruc-

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{Schematic representation of the 3D \textit{in vitro} model. Each well of the six-well plate has an insert containing the cells embedded either within the collagen or mixed Matrigel–collagen gel. Color images available online at www.liebertonline.com/ten.}
\end{figure}
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**FIG. 2.** Ductal and alveolar structures obtained in collagen gels. (A) H&E and whole mount analyses of a 5-day time course. H&Es (B) and whole mounts (C) of collagen gels with MCF10A cells and RMF in coculture as well as each cell type alone cultured for 1–6 weeks. Note the morphological polarization (inset in B) and the lumen formation (B, arrows) in epithelial structures after 2 and 3 weeks and the complex ductal network (C) that begins to form as early as 1 week of incubation. Scale bars: H&Es = 50 μm; whole mounts = 100 μm.
tions, and the mixed Matrigel–collagen gel was prepared using a 1:1 ratio of Matrigel and type I collagen keeping the final collagen concentration at 1 mg/mL. In cocultures, MCF10A and RMF were seeded in a 3:1 ratio using 300,000 MCF10A cells and 100,000 RMF. This ratio mimics the in vivo numbers of epithelial cells to fibroblasts.\textsuperscript{32} The same cell number was seeded for each cell type cultured independently. Cells were suspended in 3 mL collagen or a 3 mL Matrigel–collagen mixture and seeded into 35 mm well inserts of a six-well plate (Organogenesis) as depicted in Figure 1. The gels were allowed to solidify for 30 min at 37°C before adding combined medium onto each gel (2 mL) and each well (10 mL). Cultures were maintained for 1–6 weeks, and the medium was changed every 2–3 days.

**Gel processing**

On the day of harvest, the gels were cut into pieces. One piece was fixed overnight in 10% phosphate-buffered formalin, paraffin-embedded, and used for histological analysis. Another piece was whole-mounted onto a slide and fixed overnight in 10% phosphate-buffered formalin for morphometric analysis. The last piece was fixed overnight in 2.5% glutaraldehyde, pH 7.3, buffered using 0.1 M sodium cacodylate, and used for electron microscopy.

**Whole mount staining**

The gels that were fixed to a glass slide overnight were cut into pieces. One piece was fixed overnight in 10% phosphate-buffered formalin, paraffin-embedded, and used for histological analysis. Another piece was whole-mounted onto a slide and fixed overnight in 10% phosphate-buffered formalin for morphometric analysis. The last piece was fixed overnight in 2.5% glutaraldehyde, pH 7.3, buffered using 0.1 M sodium cacodylate, and used for electron microscopy.

**Confocal microscopy**

Whole-mounted gels were analyzed using a Zeiss LSM 510 system. The HeNE 633 nm/5 mW laser was used for data acquisition due to the autofluorescence of carmine dye at this wavelength. Alveolar and ductal structures in collagen and mixed Matrigel–collagen gels were scanned with a 20× objective lens, and 8-bit-depth images with a resolution of up to 1044×1044 pixels were taken. The data were three-dimensionally reconstructed using Zeiss software.

**Immunohistochemical analysis**

All primary antibody concentrations and supplier information are listed in Table 1. An antigen-retrieval method using microwave pretreatment and 0.01 M sodium citrate buffer (pH 6) was used.\textsuperscript{33} The antigen–antibody reaction was visualized using the streptavidin–peroxidase complex, with diaminobenzidine tetrahydrochloride (Sigma-Aldrich) as the chromogen. Counterstaining was performed with Harris’ hematoxylin. Images were captured using a Zeiss Axioscope 2 plus microscope (Carl Zeiss MicroImaging, Thornwood, NY).

**Transmission electron microscopy**

Fixed samples were washed with 0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide buffered by 0.1 M sodium cacodylate for an hour on ice. Gels were dehydrated in graded ethanol, cleared with propylene oxide, and infiltrated with a 1:1 mixture of propylene oxide and epon followed by another infiltration with a 1:2 mixture of propylene oxide and epon. Finally, the gels were infiltrated with 100% epon before embedding them in fresh epon and curing them at 60°C for 24–48 h. Following polymerization of the resin, thick sections were produced using a Reichert-Jung Ultract E microtome and stained with toluidine blue to determine orientation. The blocks were then thin sectioned at 70–90 nm and mounted onto copper grids. Grids were stained with 3% uranyl acetate and were examined at various magnifications using a Philips CM-10 electron microscope at 80 kV.

**Morphometric analysis**

Four to five experiments for each condition were analyzed, and for each experiment, three arbitrarily chosen fields at 10× magnification per section were examined. Images were captured using a Zeiss Axiovision 2 plus at 10× and 3900 dpi and analyzed with the Zeiss Axiovision version 4.4 software. The following parameters were measured per section: total number of ductal and alveolar structures, total number of cells per structure, and number of Ki67 positive cells within those structures. Proliferating epithelial cells were expressed as %Ki67 positive cells per total epithelial cell number. Morphometric analysis was carried out in collagen and mixed Matrigel–collagen gels.

**Statistics**

SPSS software package 15.0 (SPSS, Chicago, IL) was used for all statistical analyses. Independent samples t-tests were used to compare morphological parameters and proliferation index in both types of matrices and in the different culture conditions. For all statistical tests, results were considered significant at $p < 0.05$. All results are presented as mean ± SEM.

**RESULTS**

**Breast morphogenesis in collagen gels**

Cocultures of MCF10A cells and RMF. After 24 h, MCF10A cells and RMF were evenly distributed throughout the collagen gels mostly as single cells or small two to three cell aggregates (Fig. 2A). Epithelial structures began to or-
ganize after 3 days in culture, and by the fifth day, an incipient ductal network was observed (Fig. 2A).

After 1 week in culture in collagen gels, MCF10A cells formed numerous alveolar and ductal structures in the presence of RMF (Fig. 2B, C), and after 2 and 3 weeks, the ductal structures formed an elaborate network with ducts increasing in length and complexity (Fig. 2C) due to an increase in cell number that occurred mainly during the first week. At the same time, cells became morphologically polarized in both ducts and alveoli; that is, their nuclei adopted a basal location (Figs. 2B, 3A), and E-cadherin was expressed in the epithelial cell-to-cell contacts (Fig. 3A). By the fourth week, some MCF10A cells and RMF were still proliferating, and the ductal networks became more complex (Fig. 2B, C). After 4 weeks, the epithelial structures occupied the majority of the gel area, although most of the epithelial ductal cells no longer proliferated. In addition, the epithelial structures no longer resembled ductal structures, and polarity was lost (Fig. 2B, C).

Using confocal microscopy, lumina in ductal and alveolar structures were observed as early as 1 week in culture. Between 2 (Fig. 4) and 4 weeks in culture, a larger number of structures showed lumina.

**MCF10A cells grown in collagen gels.** MCF10A cells cultured alone in collagen gels showed delayed onset of organization when compared to the cocultures with RMF (Fig. 2A). MCF10A cells alone required at least 5 days in culture to become arranged into epithelial structures (Fig. 2A), indicating that fibroblasts promote the initial cell–cell communication and organization.

After 1 week in culture in collagen gels, MCF10A cells grown alone formed alveolar structures and a ductal network (Fig. 2B, C). After 2 weeks, these cells formed organized alveolar and ductal structures similar to those observed in coculture with RMF. Cells in most epithelial structures appeared polarized, and lumen formation was frequently observed in alveoli and some ducts. After 3 and 4 weeks, the ductal network became more distinct, the ducts elongated further, and the gels contained a complex ductal network with few alveolar structures (Fig. 2B, C). Similar to what was observed in coculture with RMF, after 6 weeks of incubation, abundant epithelial structures were observed. The ductal structures seemed less organized, their outlines no longer defined, and it appeared as if they had “collapsed” (Fig. 2B, C).

**RMF grown in collagen gels.** After 1 week of incubation, RMF proliferated sparsely in collagen gels (Fig. 2B, C). However, after 2 weeks in culture, the cell number increased considerably, and the cells were homogenously distributed throughout the gel. After 3 and 4 weeks in culture, RMF proliferated further. After 6 weeks, RMF maintained their homogenous distribution throughout the gel (Fig. 2B, C).

**Characterization of alveolar and ductal structures.** As mentioned above, 2-, 3-, and 4-week time points consistently resulted in ducts and alveoli resembling a normal mammary gland. To further characterize these epithelial structures, we chose the 2-week time point.

Pankeratin staining revealed that alveolar and ductal structures consisted exclusively of epithelial cells (Fig. 3A).
MCF10A cells also stained positive for keratin 18 and negative for smooth muscle actin (SMA), thus revealing their luminal character (data not shown). Further, E-cadherin staining, a marker of cell–cell adhesion, was observed in both alveolar and ductal structures (Fig. 3A). Type IV collagen staining, a marker for basement membrane, was not observed in alveolar or ductal structures regardless of whether or not RMF were present in the culture (Fig. 3A). In the cocultures of MCF10A cells and RMF in collagen gels, the number of proliferating epithelial cells, as revealed by Ki67 staining, was almost twice as high at 1 week compared to 2 weeks of incubation (p < 0.05) (Fig. 3B). When MCF10A cells were grown alone, a similar trend was observed (p = 0.09). This observation is consistent with previous studies using Matrigel, whereby once cellular polarity was established, mammary epithelial cell proliferation decreased.34 Few MCF10A cells and RMF continued to proliferate during the sixth week of incubation (data not shown). Thus, we observed a similar morphological and immunohistochemical staining pattern in cultures of MCF10A cells alone and when cocultured with RMF.

Ultrastructural analysis using transmission electron microscopy (TEM) revealed the existence of several junctional complexes such as tight junctions and desmosomes in polarized epithelial cells (Fig. 3C). However, a basement membrane surrounding the alveolar and ductal structures was not observed, which was consistent with the lack of type IV collagen staining shown in Figure 3A.

Breast morphogenesis in mixed Matrigel–collagen gels

Cocultures of MCF10A cells and RMF. As Matrigel is extensively utilized in 3D cultures of epithelial cells, we tested a matrix consisting of a mixture of Matrigel and type I collagen in a 1:1 ratio keeping the final collagen concentration at 1 mg/mL. These cultures were incubated for 1, 2, and 3 weeks. After 1 week in culture, most of the structures were alveolar and few ducts were observed in these mixed gels (Fig. 5A, B). However, the number of ducts increased with time and ductal networks started to develop after 3 weeks in culture (Fig. 5B). Further, polarized ductal and alveolar structures could be observed after 2 and 3 weeks in culture (Fig. 5A).

MCF10A cells grown in mixed Matrigel–collagen gels. MCF10A cells grown alone exclusively formed alveolar structures in the mixed Matrigel–collagen gels in contrast to the morphology observed in collagen gels (Fig. 5A, B). No ductal structures were observed at any time point analyzed. After 2 and 3 weeks in culture, the number of alveolar structures increased, but the size of each structure remained the same. Also, as early as 1 week in culture, cells in the alveolar structures were polarized; that is, their nuclei were located basally, and maintained their polarity throughout the duration of the experiment (Fig. 5A).

RMF grown in mixed Matrigel–collagen gels. In contrast to the homogeneous distribution of RMF observed in collagen gels, these cells formed clusters in mixed Matrigel–collagen gels that were often arranged into star-like shapes (Fig. 5A, B). After 2 and 3 weeks in culture, the clusters of RMF increased in size as the RMF continued to proliferate.

Morphometric analysis of collagen and mixed Matrigel–collagen gels. With regard to ductal structures there was no significant difference whether MCF10A were grown alone in collagen gels or in coculture with RMF (Fig. 6A). However, there was an increase in the number of alveolar structures in collagen gels when MCF10A cells were grown in coculture with RMF compared to when MCF10A cells were grown alone. This increase was significant after 1 week (p < 0.05) with a similar pattern persisting after 2 weeks in culture (Fig. 6B).

A 10-fold lower number of ductal structures was observed after 1 week when MCF10A cells were grown in coculture with RMF in the mixed Matrigel–collagen gels as compared to collagen gels (p < 0.05); this difference attained sevenfold after 2 weeks (p < 0.05) (Fig. 6A). In contrast, there was a significant increase in the number of alveolar structures by more than twofold after 1 week (p < 0.05) with a similar trend after 2 weeks (p = 0.053) in the mixed Matrigel–collagen gels as compared to collagen gels (Fig. 6B).
When MCF10A cells were grown alone, the number of ductal structures was significantly different after both 1 week ($p < 0.01$) and 2 weeks ($p < 0.01$) when comparing both gel types (Fig. 6A). Further, the number of alveolar structures increased significantly by more than 10-fold after 1 week ($p < 0.001$) and 2 weeks ($p < 0.001$) in culture when mixed Matrigel–collagen gels were compared to collagen gels.

Characterization of alveolar and ductal structures. The epithelial structures in the mixed Matrigel–collagen gels were characterized using the same markers as in the collagen gels. Pankeratin staining revealed that alveolar and ductal structures consisted exclusively of epithelial cells (Fig. 7A). Like in collagen gels, MCF10A cells stained positive for keratin 18 and negative for SMA, thus revealing their luminal character (data not shown). Positive E-cadherin staining provided further evidence that epithelial cells formed cell–cell junctions. Alveolar and ductal structures were able to form a basement membrane as evidenced by positive type IV collagen staining. TEM analyses further confirmed the presence of a basement membrane (Fig. 7C). This was in contrast to the results obtained with collagen gels. However, both histological and ultrastructural analyses revealed that the basement membrane was discontinuous (Fig. 7A, C).

When MCF10A cells were grown in coculture with RMF in the mixed gels, the number of proliferating cells decreased significantly between 1 week and 2 weeks in culture ($p < 0.05$) (Fig. 7B). A comparable observation was made when MCF10A cells were grown alone ($p < 0.05$) (Fig. 7B). After 2...
weeks of incubation, the number of proliferating cells in the mixed gels was similar to those found in collagen gels (Fig. 7B).

**DISCUSSION**

Here we report the characterization of a novel human breast surrogate model for the study of normal and diseased breast development in vitro while preserving the 3D architecture of the organ in vivo. This system consists of a 3D coculture of human breast epithelial cells and human breast fibroblasts embedded within (i) a defined matrix of type I collagen, that is, the major ECM component of the mammary gland, and (ii) a matrix of type I collagen and Matrigel. In the former, mostly branched ducts developed, while in the latter, alveoli and ducts were formed, highlighting the importance of matrix composition for the understanding of tissue organization. Further, this human in vitro 3D model proved to be reliable and stable for at least 4 weeks in culture.

Previously, Shekhar et al. also reported the use of a 3D coculture system of MCF10A cells and RMF. However, their reports showed disorganized epithelial clusters in which the RMF were located in the center of the cluster. These structures could not be identified either as alveoli or as ducts and did not resemble the morphology of the mammary gland in vivo. The main difference between Shekhar’s model and ours is that we embedded cells within the matrices (collagen and mixed Matrigel–collagen gels), while Shekhar et al. seeded cells onto Matrigel-coated chamber slides. In addition, Shekhar et al. used eight-well chamber slides, whereby each well had a surface area of about 1 cm², whereas our inserts have a surface of 4.67 cm². Finally, while Shekhar et al. used Matrigel only, we used either collagen or mixed Matrigel–collagen gels.

In the experiments reported herein, epithelial structures obtained in collagen gels did not form a basement membrane in contrast to those observed in mixed Matrigel–collagen gels regardless of whether MCF10A cells were grown alone or in coculture with RMF. Emerman and Pitelka reported that cells grown on floating collagen gels formed continuous basement membrane. However, these authors used primary mammary epithelial cells instead of a cell line and observed occasional myoepithelial cells in their cultures that might have been unintentionally included during the initial cell collection. Myoepithelial cells are known to secrete basement membrane components. The presence of a basement membrane surrounding alveolar structures in mixed Matrigel–collagen gels confirms observations reported when mammary epithelial cells were grown in Matrigel. Our results extend this finding by showing that the ductal structures obtained in the cocultures were also able to form a basement membrane.

We consistently observed a reduced amount of RMF when these cells were cocultured with MCF10A cells in both collagen gels and mixed Matrigel–collagen gels when compared to RMF grown alone. Even though 100,000 RMF were seeded in all of these gels, few of these fibroblasts were observed after 1 week and 2 weeks of coculture with MCF10A cells. However, in a similar experiment when skin keratinocytes were seeded together with RMF, numerous fibroblasts were observed throughout the gel even after 1 week (manuscript in preparation), indicating that MCF10A cells may selectively inhibit RMF from rapidly proliferating during the first weeks in culture. More extensive studies are needed to characterize this phenomenon.

It has been reported that mammary epithelial cells grown in rBM only form acini. The fact that in our model MCF10A cells grown alone in collagen gels were able to form ductal structures indicates that Matrigel may have inhibited these cells from elongating and forming ducts. This inhibitory effect observed in the mixed Matrigel–collagen gels was significantly diminished by the presence of RMF. This might be due to the presence of the discontinuous basement membrane observed around the epithelial structures formed in the presence of RMF, indicating that RMF play a role in inducing ductal formation by locally degrading the basement membrane. This inference is supported by the fact that during early normal development of the lung, salivary gland, and kidney, the continuous basal lamina becomes discontinuous during periods of extensive elongation and branching. Alternatively, the absence of ductal formation when epithelial cells were grown alone in the mixed Matrigel–collagen gels could be due to the different elastic properties of this matrix compared to collagen gels. As mentioned above, both matrices have different contraction properties, and physical forces play an important role in morphogenesis and cell fate.

MCF10A cells always formed ducts in the presence of RMF in both collagen and the mixed Matrigel–collagen gels. Contrary to the results reported by Tsai et al., the ductal structures that developed in our model were part of a complex network and not just isolated ducts.

Collagen gels had an approximate thickness of 5 mm at the beginning of the experiments and contracted to about 1 mm within 7–10 days when MCF10A cells and RMF were seeded in coculture. Gels containing either MCF10A cells or RMF alone contracted less rapidly but also considerably over time and reached a thickness similar to those observed in cocultures but several days later. In contrast, mixed Matrigel–collagen gels contracted only very little, if at all. Remarkably, collagen gels contracted nonhomogenously forming a thicker inner center surrounded by a thinner outer ring. We observed that cells formed different epithelial structures depending on their location within the gel. The outside ring contained more alveolar structures with long cords of epithelial cells on the extreme periphery, whereas ductal structures developed mostly in the center of the gel. Preliminary data suggest that matrix elasticity varies between the center and the periphery of the gel. The presence of diverse epithelial structures further emphasizes the importance of physical properties in tissue morphology.
structures present in the center of the gels. They represented structures similar to those observed in vivo and were observed consistently.

In the long-term task of characterizing a relevant and reliable 3D surrogate model for breast development and carcinogenesis, researchers are just now at the initial data-gathering stage of developing cocultures of two or more cell types in a mostly defined microenvironment. The two 3D cultures containing stromal and epithelial cells described herein are relevant surrogate models to study breast biology. Using these models we were able to observe ducts, which are the main structures of the resting mammary gland. The relevance of these models resides in that the majority of breast cancers are classified as ductal carcinomas. We expect that these models will allow us to perturb single components of the stroma (its cellular and ECM components) and the epithelium to assess their individual contributions to normal tissue organization, tumor formation, and its reversal.

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