Culturing Mammary Epithelial Cells in Three Dimensions

Cancer biologists are beginning to favor three-dimensional cell culture over the traditional two-dimensional (1). The context of a cell's environment is being recognized as key to signaling cell behavior, and an understanding of cell-environment interaction is a cornerstone to successful tissue engineering and creation of cell-based devices. One way to further the study of cell-environment interactions and, subsequently, the refinement of materials design is to analyze the behavior of cells in different natural and synthetic three-dimensional contexts.

Culture of Epithelial Cells in Matrigel™

The mammary gland is the basic functional unit of the milk production system of the breast. Two main cell types make up the mammary gland – the mammary epithelial cells that line the milk ducts, and the surrounding myoepithelial cells that are capable of contraction (2).

MCF-10A is a non-tumorigenic mammary epithelial cell line derived from the subcutaneous mastectomy tissue of a 36-year-old, parous, premenopausal woman with fibrocystic disease (3). When cultured on tissue culture polystyrene, the cells have a cuboidal morphology upon confluency that is typical of epithelial cells (Figure 1A). However, when cultured in the three-dimensional environment of Matrigel™ (4), the cells form hollow acini structures reminiscent of the native mammary terminal ductal alveoli (Figure 1B).

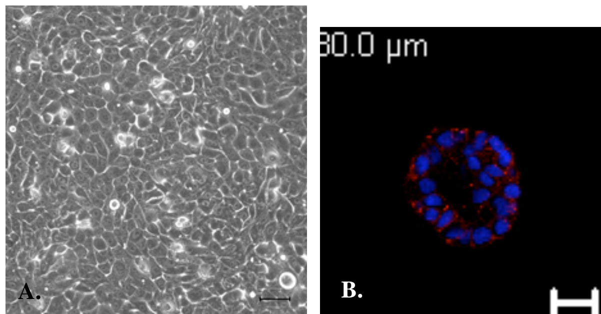

Figure 1. (A) Phase contrast microscopy of MCF-10A morphology at confluency. Bar = 100 μm. (B) Single laser scanning confocal microscopy (LSCM) optical section at approximately mid-point of a ~60um spherical acini structure formed by MCF-10A after 20 days of culture in Matrigel™. Cell nuclei are stained with TOPRO-3 (blue) and actin cytoskeleton with rhodamine-phalloidin (red). Bar = 20 μm.

Matrigel™ is an extracellular matrix extract from Engelbreth-Holm-Swarm sarcomas of mice (5). The extract is a mixture of extracellular proteins such as laminin, collagen IV and Entactin, and aside from being of mouse origin, it also contains varying concentrations of growth factors that can influence cell behavior (6). It is therefore difficult to determine how the individual Matrigel™ components—both physical and chemical—contribute to the changes in mammary epithelial acini formation.

Culture of Epithelial Cells on Electrospun Scaffold

Creating a nanofiber-based scaffold is a promising way of synthetically recapitulating the natural extracellular matrix, and electrospinning is an attractive technique for the generation of such scaffolds (7). This is because electrospinning can create non-woven mats of polymer nanofibers, which mimic an extracellular matrix in appearance and can be future modified through various post-processing techniques, such as femtosecond laser ablation, as shown in Figure 2 (10).

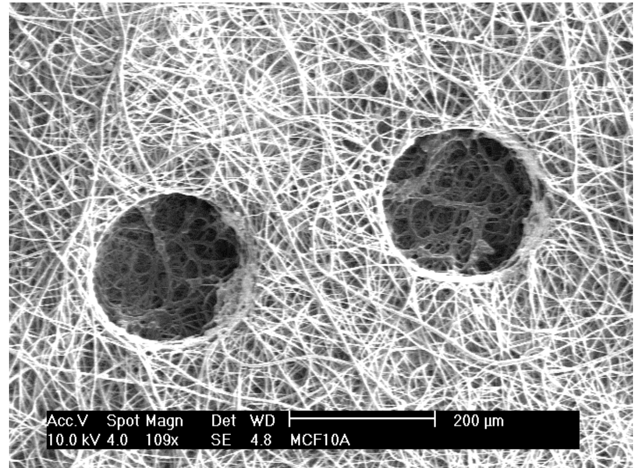

Figure 2. Scanning electron microscopy (SEM) image of electrospun poly-ε-capro-lactone (PCL) scaffold with femtosecond-laser-machined pockets for targeted cell seeding.

However, culture of MCF-10A cells on electrospun poly-ε-capro-lactone (PCL) polymer with normal culture media was not enough to promote acini structure formation, even after 20 days in culture (Figure 3).

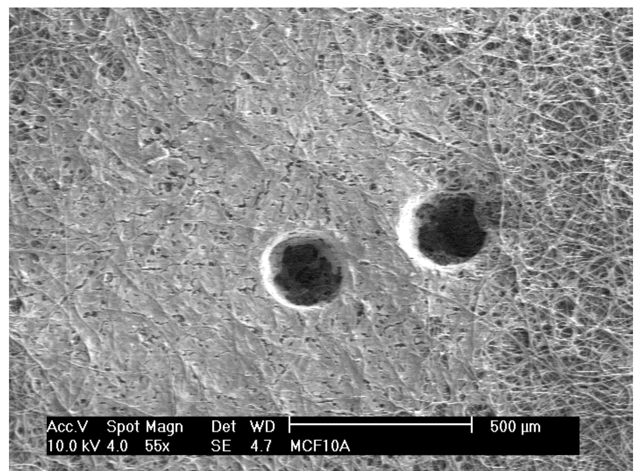

Figure 3. Scanning electron microscopy (SEM) image of electrospun poly-ε-capro-lactone (PCL) scaffold with femtosecond-laser-machined pockets that were targeted, seeded (left pocket) with MCF-10A cells and kept in culture for 20 days.

kniss.1@osu.edu
The Ohio State University, all rights reserved.

Culture of Epithelial Cells on Substrates with Matrigel™ Supplemented Media

Instead of an electrospun PCL scaffold alone, supplementation of culture media with Matrigel™ seems to be sufficient for MCF-10A mammary epithelial cells to form cell clumps that resemble the acini structure but lack the hollow lumen that is so characteristic of mammary acini.

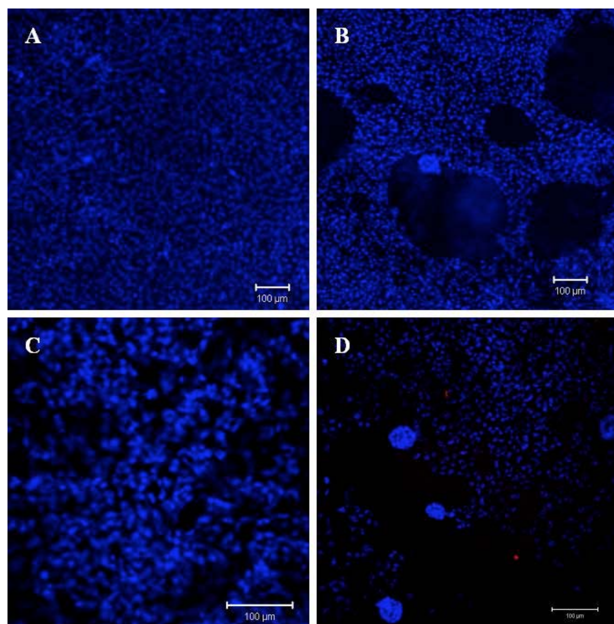

Figure 4. Fluorescent microscopy of MCF-10A culture on plain glass (A, B) and electrospun PCL scaffold (C, D) with 0% or 0.5% Matrigel™ (A and C) and 2% Matrigel™ (B and D) supplemented in the culture media. Bar = 100 µm.

As depicted in Figure 4, Matrigel™ concentrations of 0% to 0.5% in the media (A, C) do not alter the two-dimensional sheet-like growth of MCF-10A on glass (A) or electrospun PCL (C). When Matrigel™ concentration in the culture media is increased to 2%, however, cells begin to form clumps that resemble native acini structure somewhat, though lacking the characteristic acini lumen. That this phenomenon is observed both on plain glass (B) and electrospun PCL (D) substrates seems to indicate the presence of Matrigel™ is more significant in directing MCF-10A behavioral changes than the substrate upon which they are cultured.

Exploring Other Scaffolds

Work is ongoing to explore other materials as non-Matrigel™ scaffolds for MCF-10A acini formation. One substrate being explored is alginate, a natural polymer extracted from seaweed. Alginate has good biocompatibility and a simple gelation process with divalent cations, such as Ca^{2+}, and is a popular material utilized in cell immobilization and encapsulation, as depicted in Figure 5. Results thus far indicate that alginate encapsulation does not support the growth of MCF-10As nor acini formation.

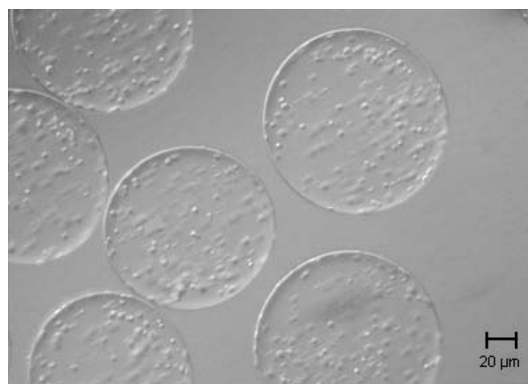

Figure 5. Encapsulated MCF-10A cells in poly-l-lysine coated, liquefied 2% alginate beads approximately ~100 µm in size. Bar = 20 µm

Other scaffolds to be tested include collagen gels; porous polymer gels made with particulate-leaching, electrospun gelatin/collagen; and electorspun coaxial PCL with gelatin/collagen outer coating.

References

1. Abbott A. Cell culture: biology's new dimension. *Nature*. 2003 Aug 21;424(6951):870-2.
2. Patrick CW. Breast tissue engineering. *Annu Rev Biomed Eng*. 2004;6:109-30.
3. Soule HD, Maloney TM, Wolman SR, Peterson WD, Jr., Brenz R, McGrath CM, Russo J, Pauley RJ, Jones RF, Brooks SC. Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. *Cancer Res*. 1990 Sep 15;50(18):6075-86.
4. Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods*. 2003 Jul;30(3):256-68.
5. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. *Semin Cancer Biol*. 2005 Oct;15(5):378-86.
6. Vukicevic S, Kleinman HK, Luyten FP, Roberts AB, Roche NS, Reddi AH. 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 Sep;202(1):1-8.
7. Boudriot U, Dersch R, Greiner A, Wendorff JH. Electrospinning approaches toward scaffold engineering--a brief overview. *Artif Organs*. 2006 Oct;30(10):785-92.
8. Zhang X, Wang W, Xie Y, Zhang Y, Wang X, Guo X, Ma X. Proliferation, viability, and metabolism of human tumor and normal cells cultured in microcapsule. *Appl Biochem Biotechnol*. 2006 Jul;134(1):61-76.
9. Zhang X, Wang W, Yu W, Xie Y, Zhang X, Zhang Y, Ma X. Development of an in vitro multicellular tumor spheroid model using microencapsulation and its application in anticancer drug screening and testing. *Biotechnol Prog*. 2005 Jul-Aug; 21(4):1289-96.

Publications

10. Choi HW, Johnson JK, Nam J, Farson DF, and Lannutti JJ. Structuring electrospun polycaprolactone nanofiber tissue scaffolds by femtosecond laser ablation. *J. Laser Appl.* 2007 19: 225.

kniss.1@osu.edu
The Ohio State University, all rights reserved.