Primary Epithelial Ovarian Cancer Cells form Spheroids when Cultured on Corning® Ultra-Low Attachment Surfaces

Customer Application Note

CORNING

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Introduction

Epithelial ovarian cancer (EOC) does not follow a traditional metastatic route via the bloodstream; instead, its spread involves exfoliation of EOC cells from the primary tumor, aggregation into multicellular spheroids, and diffusion within the peritoneal cavity (Fig. 1A). Spheroids are thought to seed the formation of secondary tumors,[1,2] causing extensive and widespread disease dissemination as well as ascites fluid accumulation. Spheroids are commonly observed in ascites fluid and are known to be resistant to chemotherapeutics,[3] possibly mediating the establishment of chemotherapeutic resistant disease in patients. EOC spheroids directly from ascites can also attach and disperse on monolayers of mesothelial cells *in vitro*,[4] recapitulating what is thought to occur on peritoneal membranes to form intra-peritoneal metastases. Based on this information, spheroids appear to be present and poised to effect metastatic spread, thus demanding further study of the molecular mechanisms mediating their formation, maintenance, and role in the establishment of secondary tumors. Such studies necessitate model systems that can recapitulate these spheroids *in vitro*, thereby facilitating the use of molecular biology techniques to mount a thorough investigation.

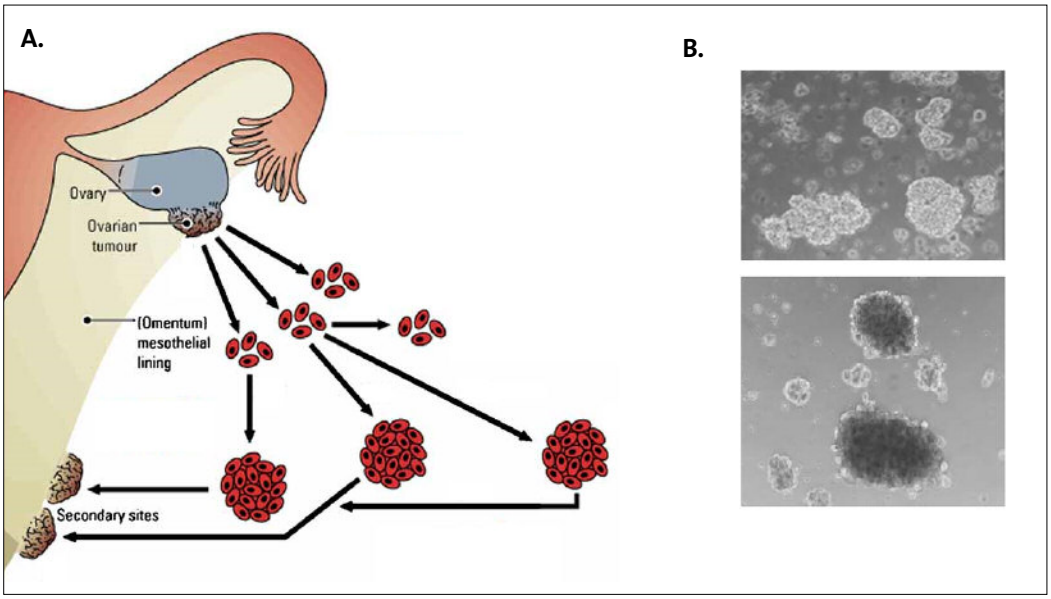

Figure 1. Spheroid-mediated epithelial ovarian cancer (EOC) metastasis. (A) EOC metastasis occurs by exfoliation of tumor cells into the fluid of the peritoneal cavity where they aggregate to form spheroids. Spheroid are thought to mediate the formation of secondary tumors (Ahmed et al., 2007). (B) EOC spheroid are often numerous and observable by brightfield microscopy. Top image: *In vivo* spheroids from patient ascites. Bottom image: *In vitro* spheroids formed with Corning Ultra-Low Attachment plates which resemble their *in vivo* counterparts.

Corning Ultra-Low Attachment Surface

To generate such a model system, our laboratory uses Corning Ultra-Low Attachment tissue culture products. Tissue culture treated polystyrene is hydrophilic and negatively charged, providing an ideal substratum for cell attachment proteins to bind. To prevent attachment, Corning® Ultra-Low Attachment products are coated with a covalently bonded hydrogel surface that is hydrophilic and uncharged, providing a non-adherent surface to which cells do not attach. No other surface we tested was able to consistently facilitate non-adherent growth of EOC cells.

Results

To generate spheroids in Ultra-Low Attachment culture, monolayer primary EOC cells cultured from patient ascites fluid were trypsinized (0.25% trypsin-EDTA solution) and seeded to Ultra-Low Attachment surfaces in media containing 10% FBS + 1% pencillin/streptomycin. By a process of aggregation and compaction,[5] EOC cells form spheroids that resemble their *in vivo* counterparts obtained directly from patients **(**Fig. 1B**)**. Other methods of spheroid formation such as hanging drop culture, spinner-flasks,[7] and forced aggregation techniques[8] can be restrictive to spheroid size and number or produce damaging shear forces. However, non-adherent surfaces provide a simple procedure with no manipulation of cells or supplementation of growth media. Spheroids formed in this way appear to be of similar size *within* an EOC patient sample. *Between* patient samples, however, mean spheroid size can differ, likely reflecting cellular properties and molecular mechanisms that are unique to each

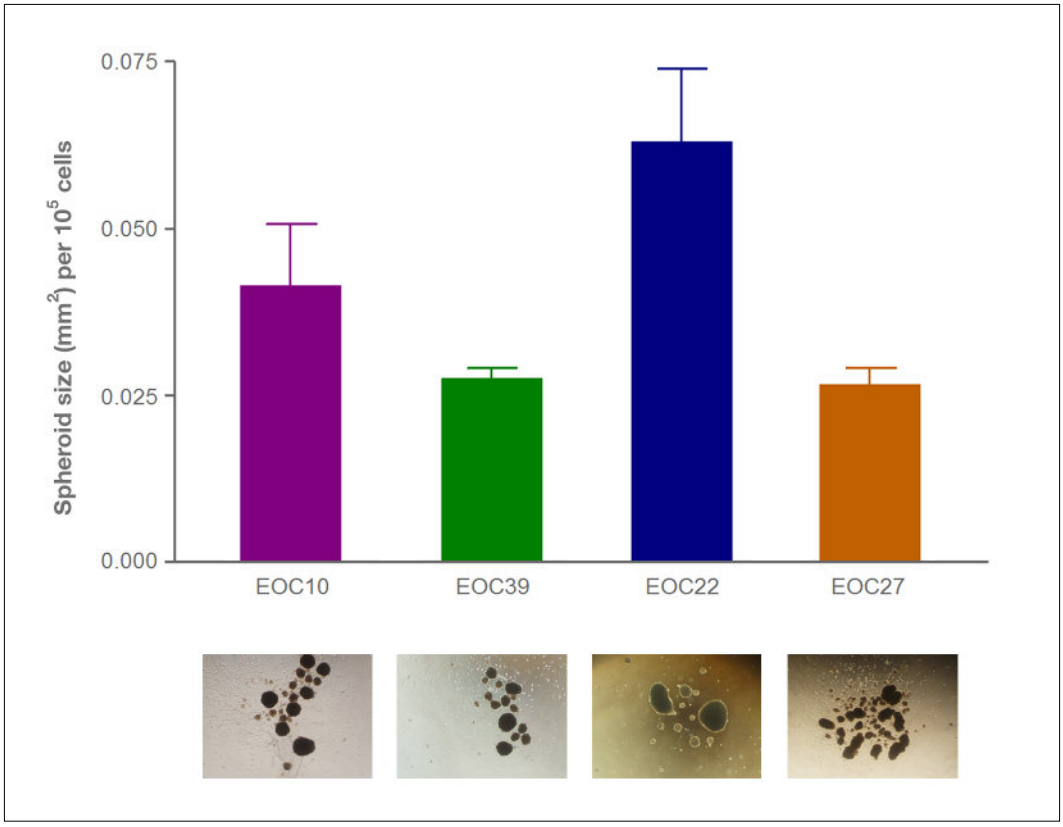

Figure 2. EOC spheroid size is consistent *within* patient samples but mean spheroid size differs *between* patient samples. 10^5 EOC cells were seeded to quadruplicate wells of a 24-well plate, incubated for 6 days, and size was quantified by surface area measurement using NIH ImageJ software. Independent EOC cells are denoted by unique patient identifiers (e.g., EOC27).

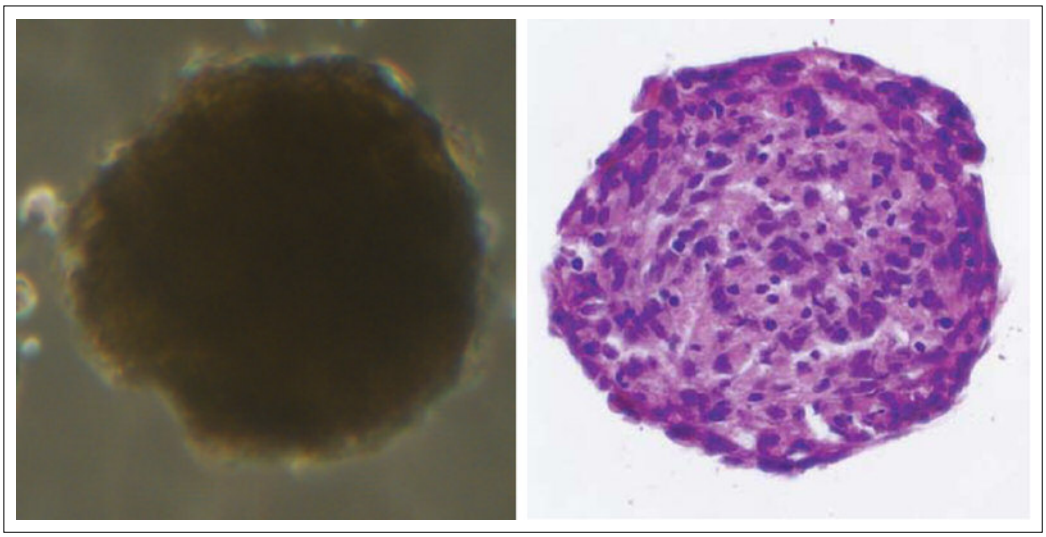

Figure 3. EOC spheroids generated on Corning® Ultra-Low Attachment surfaces can be utilized for downstream analyses. Spheroids can be sectioned and hematoxylin/eosin stained to observe internal cell morphology and permit immunohistochemical analysis.

patient sample (Fig. 2). EOC spheroids can also be fixed, paraffin-embedded, sectioned, and stained to facilitate morphologic analysis (Fig. 3). Thus, Ultra-Low Attachment plates provide an easy, convenient, and rapid means of generating biologically-relevant cell structures for the analysis of mechanisms associated with cancer cell behavior.

Prospective Research

Corning Ultra-Low Attachment products have formed the cornerstone of our endeavor to model spheroid-mediated EOC metastasis. Using spheroids formed and subsequently sectioned, we will perform immunohistochemical analyses to identify key molecules involved in the formation and maintenance of these metastatic structures. These and many other studies are possible due to the ease of use, minimal manipulation, and reproducibility afforded by Corning Ultra-Low Attachment products.

References

1. Shield, K. et al.. 2009. Gynecol. Oncol. 113(1):143-148.
2. Ahmed, N. et al. 2007. J. Cell Physiol. 213:581-588.
3. L'Espérance, S. et al. 2008. BMC Genomics 9:99-120.
4. Burleson, K.M. et al. 2004. Gynecol. Oncol. 93(1):170-181.
5. Lin, R.Z. and Chang, H.Y. 2008. Biotechnol. J. 3(9-10):1172-1184.
6. Zietarska, M. et al. 2007. Mol. Carcinog. 46(10):872-885.
7. Gilead, A. et al. 2004. Int. J. Cancer 108(4):524-531.
8. Burridge, P.W. et al. 2007. Stem Cells 25(4):929-938.

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