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REVIEW

Esophageal 3D Culture Systems as Modeling Tools in Esophageal Epithelial Pathobiology and Personalized Medicine

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SUMMARY

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The stratified squamous epithelium of the esophagus shows a proliferative basal layer of keratinocytes that undergo terminal differentiation in overlying suprabasal layers. Esophageal pathologies, including eosinophilic esophagitis, gastroesophageal reflux disease, Barrett's esophagus, squamous cell carcinoma, and adenocarcinoma, cause perturbations in the esophageal epithelial proliferation-differentiation gradient. Three-dimensional (3D) culture platforms mimicking in vivo esophageal epithelial tissue architecture ex vivo have emerged as powerful experimental tools for the investigation of esophageal biology in the context of homeostasis and pathology. Herein, we describe types of 3D culture that are used to model the esophagus, including organotypic, organoid, and spheroid culture systems. We discuss the development and optimization of various esophageal 3D culture models; highlight the applications, strengths, and limitations of each method; and summarize how these models have been used to evaluate the esophagus under homeostatic conditions as well as under the duress of inflammation and precancerous/cancerous conditions. Finally, we present future perspectives regarding the use of esophageal 3D models in basic science research as well as translational studies with the potential for personalized medicine. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.01.011)*

Q8 *Keywords:* ■■■.

Q9 Q10 Q11 The esophageal mucosa comprises stratified squamous epithelium in which esophageal epithelial cells (keratinocytes) show a proliferation-differentiation gradient and provide a barrier against the chemical and biological milieu of luminal contents. Disruption of this differentiation gradient or barrier function is linked to multiple human pathologies. Eosinophilic esophagitis (EoE), gastroesophageal reflux disease (GERD), and intestinal metaplasia (Barrett's esophagus [BE]) are benign esophageal conditions featuring aberrant epithelial cell proliferation and differentiation. In addition, adenocarcinoma (EADC) and squamous cell carcinoma (ESCC) represent the 2 primary types of malignancies arising within the esophageal epithelium and progressing via dissemination and invasion toward the underlying subepithelial stromal compartment. Three-dimensional (3D) cell culture model systems have been used as near-physiological experimental platforms to study esophageal biology under homeostatic and pathologic conditions. These 3D platforms include organotypic 3D culture (OTC) and the more recently developed 3D organoid system. In this review, we highlight the historical background of these technologies while also discussing differences among 3D culture model systems as well as applications and current limitations. Finally, we address potential future directions for these 3D model systems as they relate to esophageal epithelial biology, tumor biology, and translation in personalized medicine.

Esophageal Stratified Squamous Epithelium: Structure and Physiological Function

As a hollow muscular organ, the esophagus serves the passage of ingested food and liquid from the oral cavity to the stomach. Its luminal surface is lined by the mucosa, comprising stratified squamous epithelium and the underlying lamina propria and muscularis mucosa. The esophageal epithelium consists of proliferative basal keratinocytes and suprabasal keratinocytes, the latter undergoing postmitotic terminal differentiation, passive migration toward the luminal surface, and, ultimately, desquamation into the lumen. Through this dynamic process, a

Abbreviations used in this paper: **AKT, _____; BE, Barrett's esophagus; COX, cyclooxygenase; CSC, cancer stem cell; EADC, esophageal adenocarcinoma; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; EoE, eosinophilic esophagitis; EPC2-hTERT, _____; ESCC, esophageal squamous cell carcinoma; FEF3, primary human fetal esophageal fibroblast; GERD, gastroesophageal reflux disease; OTC, organotypic 3-dimensional culture; STAT3, signal transducer and activator of transcription-3; 3D, 3-dimensional.**

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proliferation-differentiation gradient is generated while epithelial renewal occurs over a period of 2 weeks.[1] Molecular markers defining basal keratinocytes include cytokeratins K5 and K14,[2,3] transcription factors p63,[4] and SOX2,[5] and cell surface molecules such as neurotrophin receptor p75NTR,[6] integrin β1 (CD29), integrin α6 (CD49f), and transferrin receptor (CD71).[7] Suprabasal keratinocytes are defined by differentiation markers such as cytokeratins K4 and K13, involucrin, and filaggrin,[8–10] coupled with down-regulation of basal keratinocyte markers.

Doupe et al[1] proposed that the esophageal epithelium is maintained by a single population of basal keratinocytes that give rise stochastically to proliferating and differentiating daughters with equal probability; however, functional cell heterogeneity has been postulated among basal esophageal keratinocytes. A minor subset of basal keratinocytes divide slowly or rarely and may have properties of quiescent stem cells.[11] Such a cell population may provide an explanation as to how premalignant keratinocytes accumulate genetic alterations over years without being lost through epithelial renewal. Multiple cell surface and functional markers have been suggested to identify unique subsets of basal keratinocyte stem/progenitor cells, including neurotrophin receptor $p75^{NTR}$,[6] integrins (β4, α6),[7,12] and *ABCG2* gene product.[11] Esophageal keratinocytes expressing these molecular markers have shown colony formation and self-renewal capabilities while also generating terminally differentiated progenitor cells.

Species differences exist between rodents and human beings with regard to anatomic esophageal structure. Foremost, the rodent esophagus lacks esophageal glands and papillae, both of which are present in the human esophagus. In addition, the rodent esophagus shows more explicit keratinization in the superficial cell layers, also known as *stratum corneum of the squamous epithelium*, as compared with its human counterpart. The rodent stomach consists of 2 compartments: the forestomach and distal stomach, featuring squamous epithelium and columnar epithelium, respectively, and some regard the forestomach as the counterpart of the human lower esophagus. This is important to note because Barrett's esophagus (ie, intestinal metaplasia of the esophagus) is a human mucosal lesion involving the esophagogastric junction and has been modeled at the squamocolumnar junction within the murine stomach.[13,14]

One essential physiological function of the esophageal mucosa is to serve a barrier against thermal, physical, or chemical agents, and factors related to luminal contents, including microorganisms, food antigens, gastroduodenal acids, and alcohol, all of which may contribute to the pathogenesis of esophageal diseases. Unlike the stomach, duodenum, and intestine, the luminal surface of the esophagus is not densely covered by mucus layers. Given the lack of the stratum corneum in the human esophagus and the lack of esophageal glands in rodents, the epithelial barrier function of the esophagus is attributed mainly to intercellular junctional complexes including tight junctions, adherens junctions, and desmosomes formed by cell–cell adhesion molecules such as E-cadherin, p120 catenin, and claudins. The dysfunction of these adhesion molecules has been implicated in esophageal disease conditions.[15–18]

Organ Culture and Multiple 3D Culture Models: What Are the Differences?

Throughout a long history of cell culture, various forms of 3D culture methodologies have been developed along with unique scaffolds, matrices, and cell culture media. In the esophagus, 3D culture systems have provided unique platforms to study multiple biological processes, including epithelial cell proliferation, differentiation, motility, stress response, and both homotypic and heterotypic cell–cell communications. Cellular interactions involve a variety of cell types (eg, fibroblasts, endothelial cells, and inflammatory cells) in the esophageal tissue microenvironment under homeostatic and pathologic conditions (eg, inflammatory milieu), and are mediated via cell surface molecules (eg, integrins and receptors such as Notch) as well as extracellular matrix proteins (eg, matrix metalloproteinases), as discussed in this review. The ability to experimentally manipulate 3D cultures has greatly enhanced our understanding of the molecular mechanisms and signaling pathways underlying esophageal physiology and pathophysiology.

Organ (explant) culture was a major tool for in vitro live esophageal tissue analyses before primary esophageal epithelial cell culture[19] and esophageal cancer cell lines[20] became available in late 1970s and early 1980s, respectively. The foremost advantage of organ culture is the maintenance of natural tissue architecture in situ. Organ culture may be used to study cross-talk between epithelial cells and nonepithelial cells in a live tissue-like context. Given the potential importance of a variety of cell types (ie, epithelial cells, fibroblasts, nerve cells, immune cells, and endothelial cells) present in the tissue microenvironment, organ culture indeed may be more physiologically relevant than other 3D culture systems because co-culturing multiple cell types remains difficult.

Early organ culture studies have shown that esophageal explants from animals and human beings remained viable for 3–14 days ex vivo[21] and provided substantial insight into esophageal physiological functions, including secretory and absorptive activities by basal and differentiating prickle cells,[22] as well as endocytosis mediated by prickle cells and terminally differentiated superficial cells.[23] In fetal human esophagi, organ culture detected not only epithelial cell proliferation[24] but also replacement of columnar ciliated epithelium with stratified squamous epithelium,[25] recapitulating the epithelial changes occurring during esophageal development.

Organ culture also has been used to study esophageal pathologies. Production of esophagitis-relevant cytokines was shown in patient-derived squamous epithelial explants.[26] Retinoic acid induced BE-like glandular differentiation in explants derived from squamous esophageal epithelium.[27] Explanted patient-derived mucosal biopsy specimens of Barrett's esophagus showed increased proliferation and cyclooxygenase (COX)-2 expression in response