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Review

# Engineering epithelial-stromal interactions *in vitro* for toxicology assessment

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ABSTRACT

Crosstalk between epithelial and stromal cells drives the morphogenesis of ectodermal organs during development and promotes normal mature adult epithelial tissue homeostasis. Epithelial-stromal interactions (ESIs) have historically been examined using mammalian models and *ex vivo* tissue recombination. Although these approaches have elucidated signaling mechanisms underlying embryonic morphogenesis processes and adult mammalian epithelial tissue function, they are limited by the availability of tissue, low throughput, and human developmental or physiological relevance. In this review, we describe how bioengineered ESIs, using either human stem cells or co-cultures of human primary epithelial and stromal cells, have enabled the development of human *in vitro* epithelial tissue models that recapitulate the architecture, phenotype, and function of adult human epithelial tissues. We discuss how the strategies used to engineer mature epithelial tissue models *in vitro* could be extrapolated to instruct the design of organotypic culture models that can recapitulate the structure of embryonic ectodermal tissues and enable the *in vitro* assessment of events critical to organ/tissue morphogenesis. Given the importance of ESIs towards normal epithelial tissue development and function, such models present a unique opportunity for toxicological screening assays to incorporate ESIs to assess the impact of chemicals on mature and developing epidermal tissues.

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*Abbreviations:* 2D/3D, two-dimensional/three-dimensional; ADSC, adipose-derived stromal cell; BM, basement membrane; BMP, bone morphogenetic protein; CK, cytokeratin; DPC, dermal papilla cell; EC, epithelial cell; ECM, extracellular matrix; EGF, epidermal growth factor; ESI, epithelial-stromal interaction; FGF, fibroblast growth factor; FPC, fetal pulmonary cell; HDF, human dermal fibroblast; HESC, human embryonic stem cell; HEK, human epidermal keratinocyte; HGF, hepatocyte growth factor; HIPSC, human induced pluripotent stem cell; HMVEC, human microvascular endothelial cell; HWJSC, human Wharton's jelly stromal cell; IGF-1, insulin-like growth factor-1; MESC, mouse embryonic stem cell; MMP, matrix metalloproteinase; MSC, multipotent stromal cell; ORSK, outer root sheath keratinocyte; SHH, sonic hedgehog; SMG, submandibular gland.

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## 1. Introduction

Epithelial cells actively promote tissue function by serving as a protective and responsive barrier that regulates molecular transport or by secreting specialized macromolecules into blood stream or ducts. Epithelial cells (ECs) exhibit a range of different morphologies (*e.g.* squamous, cuboidal, columnar) characteristic of their phenotype and function in the tissue milieu. For example, squamous alveolar ECs are specialized to secrete surfactants and provide a thin, protective, and selectively permeable barrier between atmospheric air and the pulmonary circulation (Ward and Nicholas, 1984). Cuboidal ECs can form hollow lumens and are specialized to secrete endocrine hormones into the blood stream or exocrine products into ducts (Hassiotou and Geddes, 2013; Holmberg and Hoffman, 2014). A critical aspect of epithelial tissue function is active crosstalk between ECs, the cell microenvironment, and the tissue stroma.

Reciprocal epithelial-stromal interactions (ESIs) direct tissue morphogenesis during development and regulate homeostasis in adults (Cunha et al., 1985, 2004). ESIs broadly consist of biochemical and biophysical crosstalk between stromal cells (*e.g.* fibroblasts, endothelial cells, smooth muscle cells, adipocytes), the extracellular matrix (ECM), and ECs, and promote normal epidermal (*e.g.* skin, cornea, tooth, mucosa) and glandular (*e.g.* mammary, pancreas, salivary, lacrimal) tissue development and function. ESIs can also contribute to tumor growth and metastasis (Parrinello et al., 2005) and disease progression (Strand and Hayward, 2010) as discussed in other reviews. During development, fusion of the neural tube, cardiac endocardial cushions, palatal shelves, and urethral folds are all critically dependent on ESIs (Ray and Niswander, 2012). These developmental processes are sensitive to perturbation with environmental contaminants that result in birth defects, and this motivates the need for robust models of developmental morphogenesis *in vitro*.

Culture models of developmental processes currently lack the throughput and human developmental relevance needed for high throughput toxicity assessment. Historically, tissue recombination, trans-well, and conditioned medium assays have all been used to study ESIs *in vitro*. For example, recombination of mammalian epithelial and stromal tissues has contributed to understanding the importance of fibroblast growth factor-10 (FGF-10)/sonic hedgehog (SHH) crosstalk (Rice et al., 2004) and epidermal growth factor (EGF) signaling (Tyler and Pratt, 1980) during embryonic palate fusion. Tissue explant studies are inherently low throughput and cannot fully recapitulate the relative contribution of signaling pathways that underlie human developmental processes (Fougerousse et al., 2000). In comparison, trans-well and conditioned medium assays can be used to interrogate paracrine signaling between epithelial and stromal cells *in vitro* (Miki et al., 2012). However, these assays are fundamentally limited by dilution of paracrine factors and depletion of nutrients in the conditioning phase that are needed by the secondary cell type. Trans-well and conditioned medium assays also cannot recapitulate epithelial tissue architecture, wherein stroma and epithelium are separated only by a basement membrane (BM) that promotes cell adhesion and regulate ESIs (Yurchenco, 2011). In contrast, three-dimensional (3D) culture of stromal and ECs (Fig. 1) can support tissue morphogenesis by recapitulating the tissue architecture and mass transport properties essential to tissue morphogenesis (Shamir and Ewald, 2014). The purpose of this review is to introduce the reader to bioengineering strategies that have been used to incorporate ESIs into models of epidermal and glandular tissues using primary cells and stem cells and to emphasize the importance of incorporating ESIs into toxicological screening assays. Finally, we derive insights into how engineered ESIs could enable the design of embryonic tissues *in vitro* that would enable the assessment of developmental morphogenesis for toxicology applications.

## 2. Layer-by-layer assembly to engineer tissues that promote ESIs

### 2.1. Skin tissue engineering

Skin is the foremost example of an adult tissue with which a bottom-up approach has been taken to promote ESIs and functional tissue formation *in vitro*. Skin tissue function is dependent on ESIs between epidermal keratinocytes, squamous epithelia, and BM separating the epithelium and underlying dermis containing ECM proteins (primarily collagens) and dermal fibroblasts (Fig. 1B) (Werner et al., 2007). Skin tissue engineering approaches can provide important insights into the materials, cell types, and methods (Table 1) that promote ESIs and normal epithelial tissue phenotype and function.

Human skin equivalents are commonly produced using layer-by-layer assembly (Fig. 1A) of encapsulated or monolayer stromal cells with a juxtaposed monolayer of keratinocytes to recapitulate the stratified nature of mature skin tissue. Typical 3D culture approaches involve encapsulating cells by mixing them in a soluble ECM that can solidify upon a stimulus to form a hydrated matrix with embedded cells. For example, skin equivalents comprising human epidermal keratinocytes (HEKs) seeded on gelatin-chondroitin-6-sulfate-hyaluronic acid hydrogels (Wang et al., 2006), fibrin (Flasza et al., 2007) or BioReagent-coated collagen (Kinsner et al., 2001) with encapsulated human dermal fibroblasts (HDFs) maintained epithelial and stromal phenotype and skin-like tissue stratification *in vitro* (Table 1). Similar skin equivalents using collagen I (Casasco et al., 2001; Eaglstein et al., 1995; Mirastschijski et al., 2006), collagen-glycosaminoglycan (Cooper et al., 1993), plastic compressed collagen (Hartmann-Fritsch et al., 2013), or decellularized amniotic membrane (Sanluis-Verdes et al., 2015; Yang et al., 2009) successfully engrafted to immune-compromised mice, demonstrating their biocompatibility. This approach is contrasted by traditional 2D cell culture techniques wherein cells are attached to a substrate on only one side. For example, HEKs and HDFs seeded on opposite sides of decellularized dermis (Ojeh and Navsaria, 2014) or collagen-glycosaminoglycan-chitosan (Black