“Increasing complexity at all costs – but why?”

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Traditionally, in vitro cell culture models have relied on single-cell-type systems, typically cultured in submerged conditions within well formats ranging from 6-well to 96-well plates. In recent years, however, the development of more complex models involving multiple cell types has attracted scientific and regulatory interest. Notably, the cultivation of lung cells at the air-liquid interface (ALI) has introduced a new level of complexity to *in vitro* systems.

Such advanced models demand rigorous characterization of the properties of the cell types involved, particularly as culture conditions become more complex in respect to media and growth factos used. The behavior and properties of cells can undergo substantial changes when cultured in co-culture, as gene expression patterns and functional responses are strongly influenced by the interactions between cell types and culture conditions such as ALI. Thus, it becomes essential to evaluate not only the characteristics of individual cell types but also the dynamic changes that occur in co-culture systems.

Furthermore, it is crucial to understand both the commonalities and differences between 3D *in vitro* models, human *in vivo* tissues, and the animal models previously used in research.

Ideally, *in vitro* models should achieve a balance by being as complex as necessary to accurately replicate physiological responses while remaining practical for economical reasons and their experimental use.