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### Functional Immortalization for the Generation of Personalized Cell Systems

A major limitation of current research is the shortage of functional personalized cells. To generate cells in sufficient numbers, in vitro cell expansion is an attractive alternative. Cell expansion can be achieved by expression of immortalization genes. Establishing new cell lines is, however, an unpredictable and cumbersome process. In addition, conventional immortalization regimens often lead to drastic alterations of cellular physiology.

To overcome these limitations, we established a novel cell immortalization approach to reproducibly generate functional and immortalized cell lines. This approach relies on a custom library of more than 30 immortalization genes and allows efficient and reproducible creation of novel cell lines. We used this process to reproducibly establish cell lines from more than 20 different cell types and different individuals.

Single cell lines were generated within 2-3 months and included e.g., endothelial cells, astrocytes, smooth muscle cells, chondrocytes, kidney epithelial cells, thyroid epithelial cells, lung and intestinal epithelial cells. Resulting cell lines are immortalized and can be cultivated for more than 100 cumulative population doublings. They can be easily cryopreserved and are amenable to genetic engineering. Functional characterization demonstrated that the novel cell lines maintain a primary-like phenotype and show cell type-specific functions.

Once an immortalization regimen was established for a certain cell type, it could be easily adapted for the reproducible immortalization of the same cell type from a different donor paving the way for individualized, patient-specific cell lines.

Novel cell lines responded to 3D culture conditions (e.g., matrix-free spheroids, Air-Liquid-Interface, semipermeable membrane) with an improved overall phenotype demonstrating their suitability as a reproducible and functional cell source for microphysiological systems.

This novel immortalization approach reproducibly provides personalized cell systems in sufficient numbers for even the most demanding applications.

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