

Human organo-typical co-culture models

EDI-CO gut:

Functionally differentiated intestinal epithelium together with whole-blood culture

The human cell line CaCo-2 (colon carcinoma derived) is well known to form a fully differentiated and functional epithelial layer, used e.g. for bioavailability studies in accordance to FDA regulations. CaCo-2 cells readily grow and differentiate on porous membranes of transwell® inserts. This allows to use the trans-epithelial electrical resistance (TEER) between the upper compartment (“luminal”) and the lower one (“baso-lateral”) as a measure of barrier function. Another parameter for judging differentiation is the formation of domes (see SEM photography, Fig. 1).

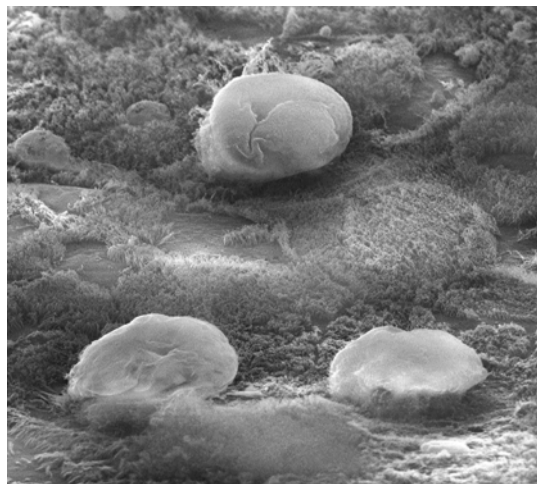
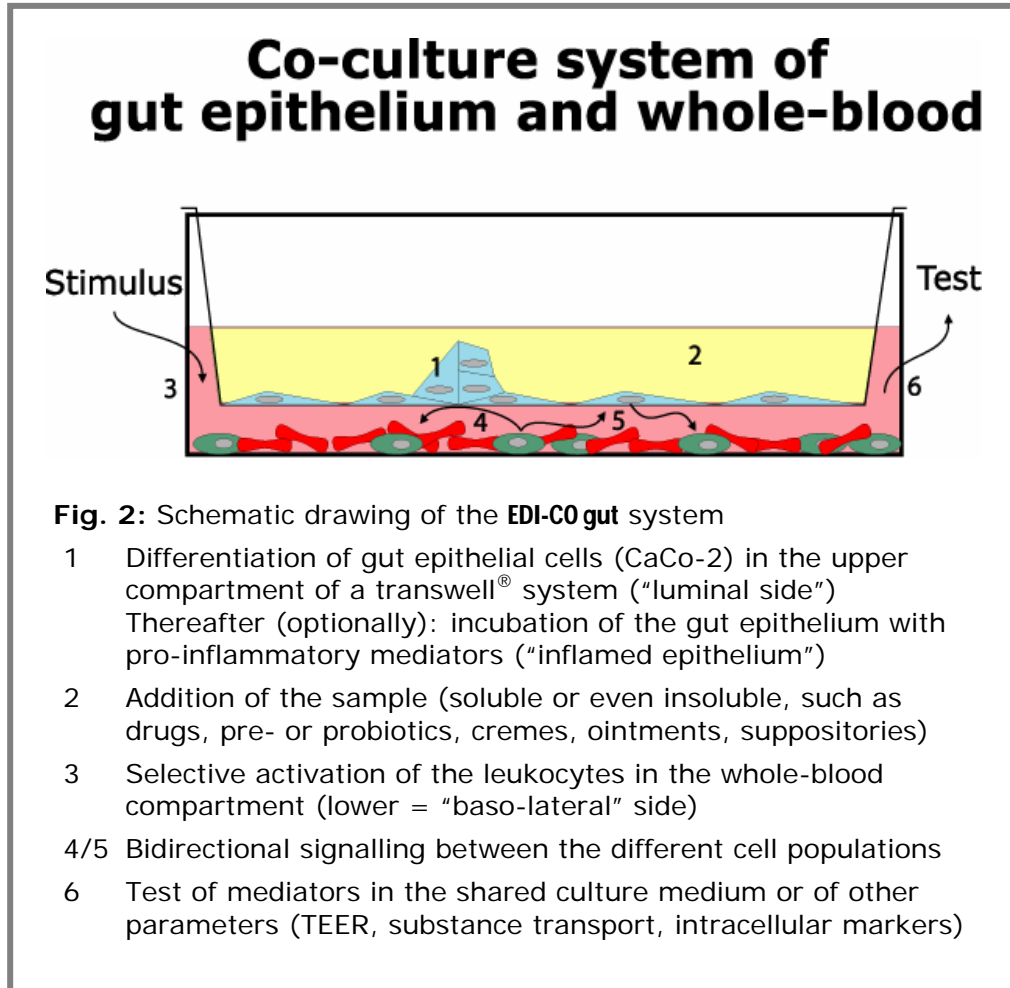


Fig. 1: SEM picture of a differentiated intestinal cell layer on transwell® inserts

The lower compartment consists of a particular human whole-blood culture system (see Fig. 2). The major advantage of the latter - in contrast to the more common PBMC (peripheral blood mononuclear cell) cultures - is that whole-blood cultures provide a much more complete and precise representation of the cross-talk between all different cellular as well as non-cellular elements of the human immune system. Furthermore, all cellular activation takes place in a physiological environment (artificial stimulation by plastic adherence is eliminated).

Only soluble products can pass the membrane between the two compartments (pore size: 0.4 μm). There is no direct contact of the cells in this co-culture model. The signalling cross-talk therefore includes the transport of drug substances as well as the secretion of mediators on both sides, the gut epithelium as well as the leukocyte compartment.



Supernatants and/or cell contents are collected at the end of culture to test for various parameters such as:

Cell type	Cytokines	Chemokines	Others
Granulocytes	–	–	Elastase
Monocytes	IL-1 β , IL-6, IL-12 TNF α , TGF β etc.	IL-8, MCP-1, etc.	MMPs
T-lymphocytes	IL-2, IL-4, IL-5, IFN γ etc.	IL-8, MCP-1, RANTES, etc.	–
B-Lymphocytes	IL-10	–	–
Epithelial cells	–	IL-8	TEER, defensin, substance transport

Also **multiplex testing for pattern recognition** of more than 20 different parameters from the same co-culture is possible.

Organo-typical conditions to investigate drug effects on human tissues can be obtained e.g. by combining differentiated human gut epithelia together with human whole-blood cultures in a two-chamber culture model.

An additional specific development of EDI was the construction of a particular applicator that avoids mechanical damage of the differentiated CaCo-2 cell layer during the addition of solid drug samples. So this new co-culture system can also be used to screen for pharmacological effects not only of soluble pharmaceuticals but also of solid probes (like suppositories, cremes, etc. or even viable probiotic bacteria, see Fig. 3).

EDI-CO gut: Sample results

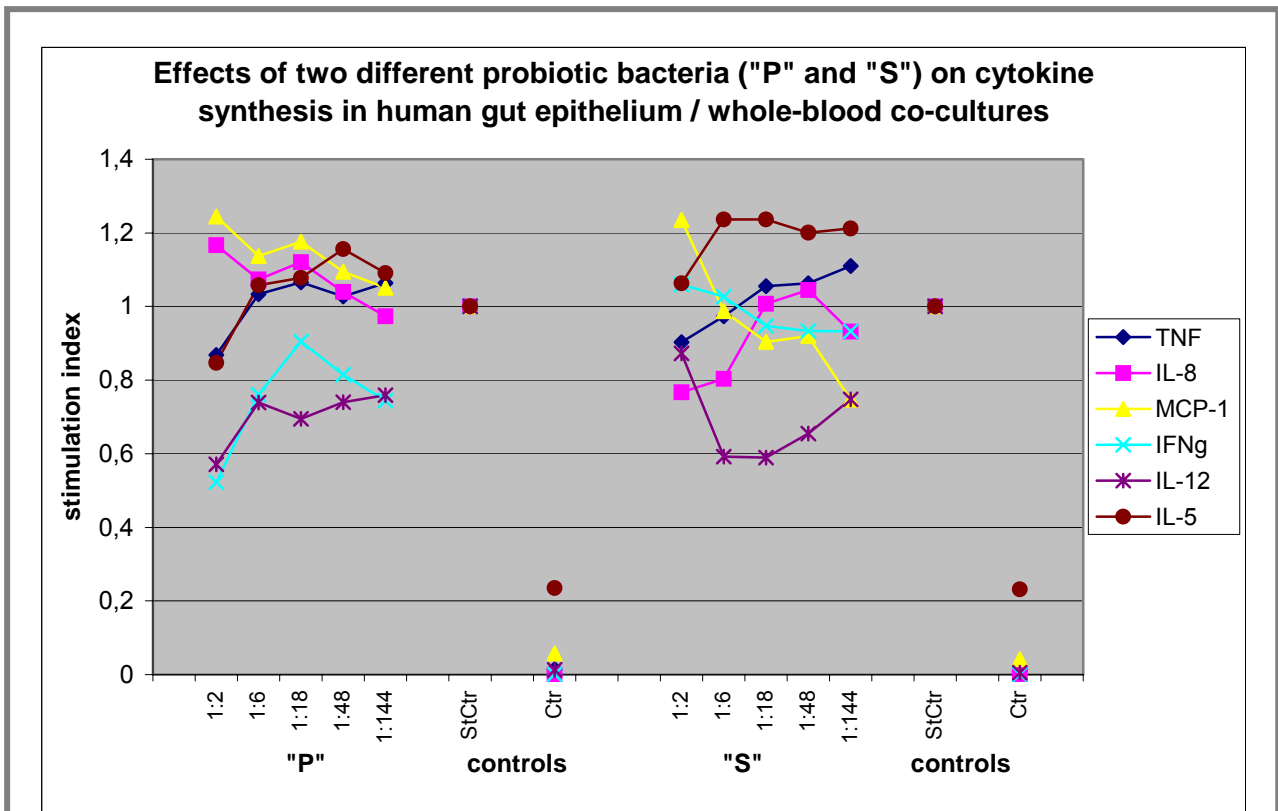


Fig. 3: Two different probiotic bacterial preparations were tested in co-cultures. Whole-blood leukocytes were activated after co-incubation with gut-epithelia that were loaded with probiotic bacteria on their luminal surface. Abbreviations: StCtr = stimulation control; Ctr = non-stimulated control.