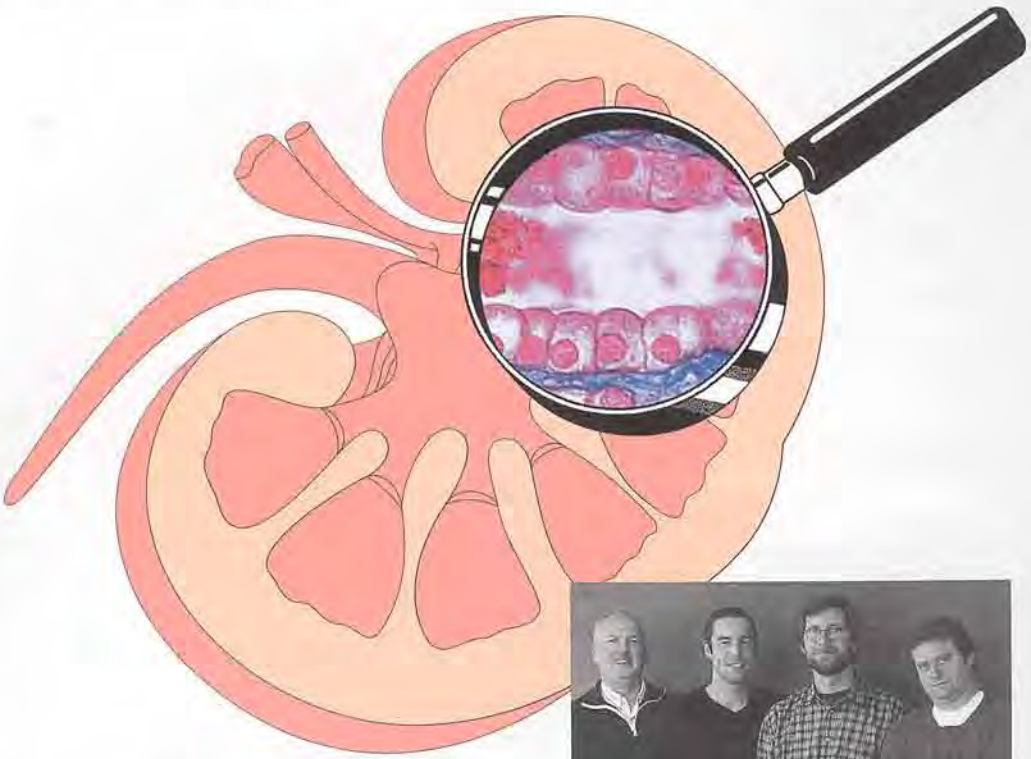
Epithelia – Biomaterials – Tissue Engineering

Epithelia form functional barriers within our organism and are exposed to different environments on their luminal and basal side. This important aspect must be considered when testing new biomaterials as well as in tissue engineering. In order to obtain realistic information on interactions between epithelia and artificial matrix under in vitro conditions, tissues must be exposed to physiological mechanical and rheological stresses for an extended period of time. To avoid damage to the epithelial barrier function leaks or pressure differences within the culture system must be avoided. In addition the cellular environment must be designed in a way to support specific function of the cultured tissue and to avoid cellular dedifferentiation. We have been developing improved culture methods for tissue culture because these experiments cannot be performed in conventional culture dishes.



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New challenges: Biomaterial research and tissue engineering

Besides the broad field of connective tissue engineering such as cartilage and bone [1] there is growing interest in epithelia. Today's goals are the generation of perfect skin equivalents [2,3], vessel implants [4,5,6], insulin-producing organoids [7,8], liver- [9,10] and kidney-modules [11,12] as well as the development of urinary bladder [13], esophagus [14] and trachea constructs [15]. Biomedical application of these artificial tissues will only be successful if the epithelia maintain the necessary degree of functional differentiation and form a close functional bond with their supporting artificial matrix. The artificial matrix which provides mechanical stability to the construct and the living tissue influence each other. It is essential to evaluate in vitro how well epithelial cells attach to the supporting matrix and how long they can withstand a functional load while maintaining specific barrier and transport function.

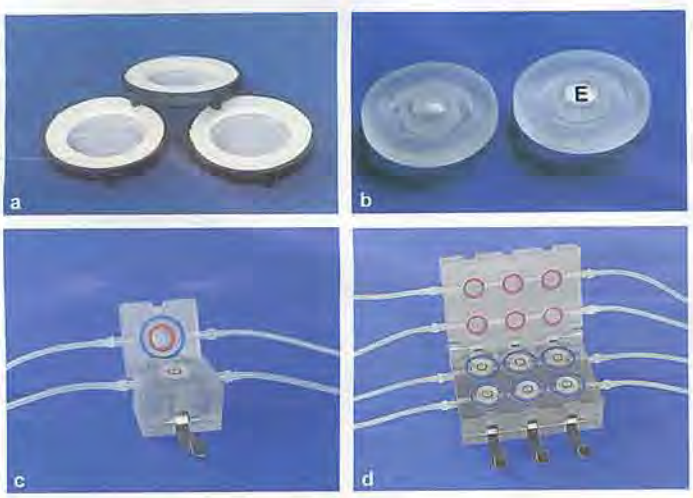

Fig. 1 a-d: Tissue carriers and gradient perfusion containers. a) Tissue carriers can hold any artificial matrix (13 mm diameter). b) Modified tissue carriers for collagenious membranes. c) Gradient culture container for 1 tissue carrier d) Gradient culture container for 6 tissue carriers. Luminal and basal compartment can be perfused individually.

Innovative culture techniques

While there is a variety of efficient techniques for the expansion of cells in culture, fundamentally new methods are required for the genera-

Keywords

Epithelium, Biomaterial research, Tissue engineering, Gradient perfusion culture

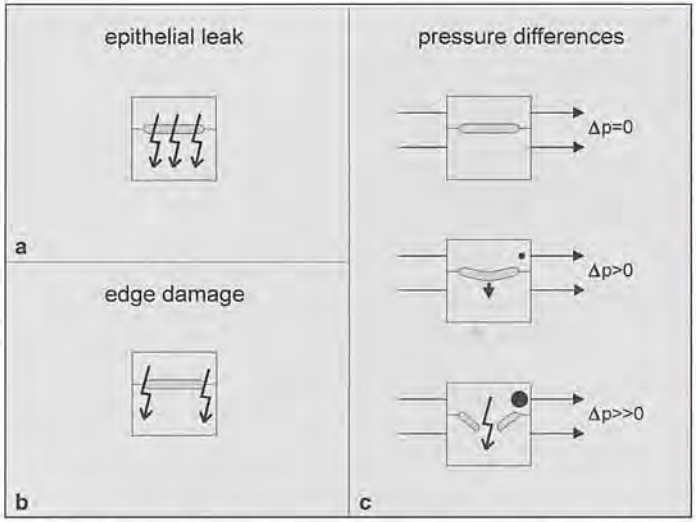

Fig. 2 a-c: Schematic representation of problems associated with gradient culture of epithelia. a) Epithelial leaks are caused by insufficient contact between neighboring cells. b) Edge damage can occur where living tissue comes in contact with the tissue carrier or artificial extracellular matrix material. c) Pressure differences can disrupt the epithelial barrier function. Optimally there is zero pressure difference between both compartments (Δp=0). Pressure differences can occur due to the unilateral formation of gas bubbles (small black dot in flow path) causing a reversible protrusion of the epithelium towards the lower pressure compartment (Δp>0). Formation of a larger embolus (large black dot in flow path) and increasing pressure differences eventually result in the disruption of the epithelium (Δp>>0).

tion of tissue constructs [2-15]. Little is known about how embryonic cells develop into a mature epithelium. Experiments show that a combination of factors including the extracellular matrix and ion milieu exerts a directional influence on development [16]. In order to imitate these influences in vitro new tissue specific culture techniques become necessary.

Tissue carriers (Fig. 1a,b) which hold a large variety of filters or membranes to seed epithelial cells onto [16,17] are placed into gradient culture containers. The containers are separated into a luminal and a basal compartment by the epithelium developing on the carrier. Both compartments can be perfused individually to create individual environments for embryonic and mature epithelia.

Epithelia under permanent stress

A common problem in these culture experiments is the incomplete development or loss of the epithelial barrier function during culture. Epithelial leaks (Fig. 2a) can be caused by a lack of cellular confluence, uneven geometrical distribution or insufficient contact between neighboring cells. Edge damage (Fig. 2b) is found in places of increased mechanical stress where living tissue, tissue carrier and artificial matrix come in contact with each other [18].

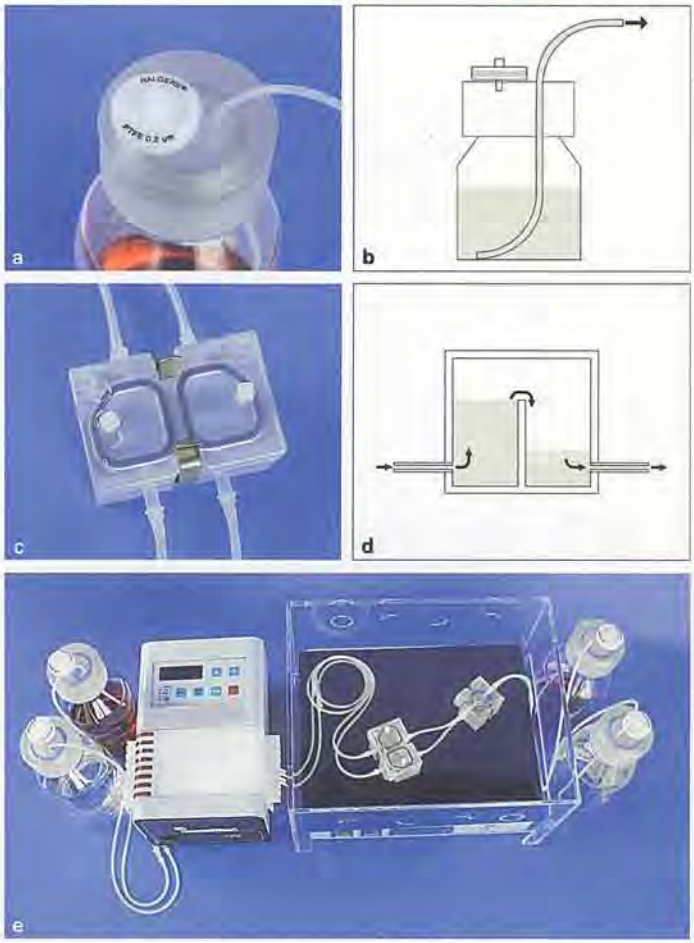

Fig. 3 a-e: New developments to minimize bubble formation in gradient culture experiments. a, b) Specially designed bottle caps. Medium can be drawn from the bottle through silicon tubing avoiding all contact with cap material. c) Gas expansion module to eliminate gas bubbles. d) During the passage through the gas expansion module the medium passes over a barrier forcing gas bubbles to separate from the liquid phase.

Pressure differences between luminal and basal compartment can lead to a disruption of the epithelial integrity (Fig. 2c). Under perfect conditions there is no pressure difference between basal and luminal compartment (Fig. 2c; Δp=0). During passage from the storage bottle to the gradi-

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IMDM + NaCl luminal	**Na^+**	**mmol/l**	**130,0**	**129,7**
	K^+	mmol/l	4,01	3,93
	Cl^-	**mmol/l**	**91,5**	**91,0**
	Ca^{++}	mmol/l	1,11	1,11
	Osmolarität	**mOsm**	**275**	**275**
	pH		7,4	7,4
	pO_2	mmHg	193,7	191,6
	pCO_2	mmHg	10,7	6,2
	Glukose	mg/dl	443	443
	Lactat	mmol/l	0	0
a	Phenol Rot		+	+
IMDM basal	**Na^+**	**mmol/l**	**117,7**	**117,9**
	K^+	mmol/l	3,96	3,96
	Cl^-	**mmol/l**	**79,8**	**80,4**
	Ca^{++}	mmol/l	1,15	1,15
	Osmolarität	**mOsm**	**253**	**253**
	pH		7,4	7,4
	pO_2	mmHg	191,8	191,6
	pCO_2	mmHg	11,9	6,5
	Glukose	mg/dl	446	445
	Lactat	mmol/l	0	0
b	Phenol Rot		-	-

Fig. 4: Physiological parameters of a gradient culture experiment after 10 days. Culture medium was analyzed before and after the culture container in the luminal and in the basal flow path. A gradient of luminal 130 mmol/l Na vs. basal 117 mmol/l Na is maintained throughout the culture period demonstrating the epithelial integrity.

ent container culture media are saturated by diffusion of atmospheric gases through the tubing. Problems arise when gas bubbles accumulate unilaterally within the tubing to form an embolus leading to a unilateral increase in hydrostatic pressure. Small pressure differences cause a reversible protrusion of the epithelium (Fig 2c; $\Delta p>0$). Increasing pressure differences eventually result in the disruption of the epithelium (Fig 2c, $\Delta p>>0$).

Environment

To avoid pressure differences the formation of gas bubbles needs to be minimized. For physical reasons bubbles preferentially form where different polymer-materials come in contact with each other. Therefore special bottle caps (Fig. 3a) were developed that allow the culture medium to be drawn from the bottle through silicon tubing avoiding all contact with cap or connector material (Fig 3b). Gas bubbles are further reduced by inserting a specially designed gas expansion module in series before the gradient culture container (Fig. 3c).

Medium parameters in gradient perfusion experiments are monitored throughout the 14d culture period (Fig. 4) using a Stat 9 Plus Analyzer (Nova Biomedical) in 500 µl samples drawn from the luminal and basal side, before and after the gradient culture container.

All culture media are buffered with HEPES (Gibco BRL) or Buffer-All (Sigma) to maintain a constant pH of 7,4 throughout the whole culture period. Analysis before the

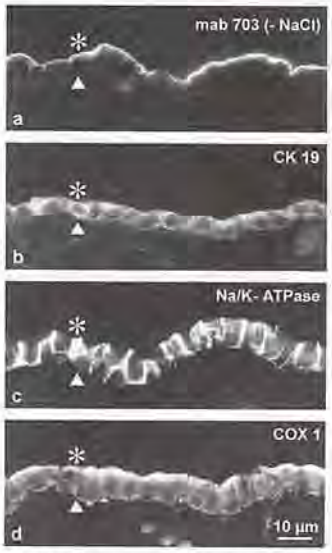

Fig. 5 a-d: Immunohistochemical differentiation profile of embryonic collecting duct epithelium after 14d in gradient culture. a) All cells are positive for the tissue-specific mab 703 . b) All cells are positive for cytokeratine 19 c) All cells show basolateral staining for Na/K ATPase. d) All cells are positive for COX1.

container shows a CO_2 partial pressure of only 11–12 mmHg due to the low CO_2 content in room atmosphere. The high O_2 partial pressure of 191 mmHg on the other hand results from diffusion of oxygen through the gas permeable silicon tubing. The continually high glucose and low lactate measurements obtained after the container demonstrate that the medium exchange is sufficiently high not to impair aerobic processes.

By using differently colored media on the luminal and basal side epithelial integrity could easily be monitored by checking for a clear color separation of the media throughout the culture period. The different electrolyte content of the culture media (e.g. 130 mmol/l luminal vs. 117 mmol/l basal Na) could be used as an additional control in our experiments. Comparing luminal and basal electrolyte content before and after the container was a sensitive indicator of epithelial integrity.

Tissue Modulation

The tissue shown here is an embryonic epithelium from the mammalian kidney, exposed to a NaCl gradient under serum-free culture conditions. The degree of cellular differentiation was examined immunohistochemically (Fig. 5). Under NaCl loaded conditions all cells are positive for the monoclonal antibody (mab) 703 (Fig. 5a), cytokeratine 19 (Fig. 5b), Na/K ATPase (Fig. 5c) and COX1 (Fig. 5d). In controls less than 10 % of the cells are positive for mab 703. These experiments demonstrate the great influence the electrolyte environment can exert on differentiation [19].

Summary

This report deals with experimental difficulties that arise when culturing epithelia in a fluid gradient. Problems are most often caused by gas bubbles leading to hydrostatic pressure differences. As a countermeasure new bottle caps and gas expansion modules were designed allowing a drastic reduction of bubbles without decreasing oxygen content. The aspects discussed in this article are of special concern for all long term culture experiments in pharmacological and biomaterial testing [20].

Tissue culture products development and sales: Minucells and Minutissue GmbH
www.minucells.de

Literature

A list of references can be obtained from the author upon request.

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