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Leading Opinion

A modular culture system for the generation of multiple specialized tissues☆

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ABSTRACT

Numerous factors influence cell functions and tissue development in culture. A modular culture system has been developed to allow the control of many of these important environmental parameters. Optimal adhesion of cells is obtained by selecting an individual biomaterial. Selected specimens are mounted in a tissue carrier in order to protect it against damage during handling and after seeding cells, the carriers can be used in a series of compatible perfusion culture containers. This technique allows the simple bathing of growing tissue under continuous medium transport and the exposure of epithelia to a gradient with different fluids at the luminal and basal sides. A further container is made of transparent material to observe microscopically the developing tissue. In addition, a special model features a flexible silicone lid to apply force to mimic the mechanical load required for developing connective and muscular tissue. Perfusion culture of stem/progenitor cells at the interface of an artificial interstitium made by a polyester fleece results in the spatial development of tubules. During long term culture over weeks the growing tissue is continuously exposed to fresh nutrition and respiratory gas. The medium is transported in a constant flow or in pulses, preventing unstirred layers of fluid. A variety of applications of this modular system, described in this paper, demonstrates that the biological profile of cells and tissues can be strongly improved when perfusion culture with a permanent provision of fresh medium is applied.

1. Introduction

The conditions of cell cultures for the study of cellular interactions with innovative biomaterials in the development of new strategies for therapeutic regeneration of epithelial, connective, muscular or nervous tissue and the investigation of cellular functions under the influence of newly developed pharmaceuticals are extremely important. While the expansion of isolated cells in the static environment of a culture dish poses no great difficulties, developing tissues especially in combination with biomaterials often show severe morphological, physiological and biochemical changes caused by dedifferentiation [1–8]. This process is not due to a single factor, but is highly dependent on the selected biomaterial, cellular adhesion, intercellular communication and culture conditions such as nutrition, adapted respiratory gas or individual rheological stress [9–14]. Since all these factors have to complement one another advanced techniques are needed to offer individual culture environments supporting tissue-specific development.

According to the multitude of factors governing cell and tissue development a system is presented here that adjusts the culture conditions to many specific needs. The concept is based on tissue carriers including selected biomaterials for an optimal cell adhesion and communication (Figs. 1–3). In different perfusion containers an individual fluid environment can be simulated improving functional differentiation of cultured cells and tissues (Figs. 4 and 5). The modular system provides a highly flexible basis for the culture of cells and the generation of multiple highly specialized tissues. On the other hand the presented system bridges a methodical gap between frequently used static cell culture, advanced tissue culture and modern microreactor technology. All of the tools are designed for multiple uses and can be sterilized in an autoclave.

2. Selecting a biomaterial support system

One prerequisite for optimal cell and tissue development in the organism is a positive interaction with the extracellular matrix. Under in vitro conditions a selected biomaterial replaces the natural extracellular matrix. This may influence the development of important functional features in a good or bad manner. Culture experiments have elucidated that a variety of different biomaterials

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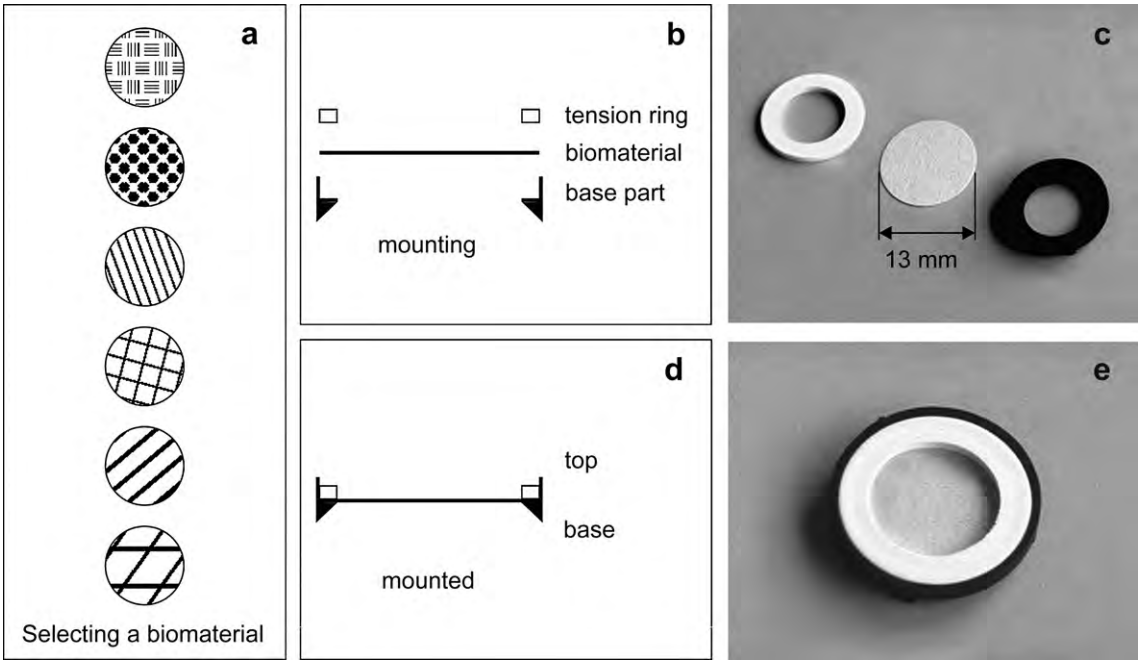


Fig. 1. Schematic illustration of mounting a Minusheet® tissue carrier. (a) First an individual biomaterial supporting optimal cell development is selected. (b,c) For mounting the biomaterial measuring 13 mm in diameter is placed in the base part of a tissue carrier and fixed by a tension ring. The base part of a mounted tissue carrier rests with protrusions at the bottom of a dish. (d) Vertical view and (e) view to the surface of a mounted tissue carrier.

such as synthetic polymers, decellularized matrix, biodegradable scaffolds [15–21], ceramics [22–24] or metals [1] may act as suitable substitutes for the extracellular matrix. Such biomaterials occur in form of filters, nets, foils, fleeces or foams to investigate their influence on growth and differentiation of cells and tissues during culture.

To stay compatible with a 24-well culture plate selected biomaterials can be punched out to a diameter of 13 mm (Fig. 1a). In order to prevent damage during handling and growth the selected specimen is then transferred to the base part of a Minusheet® tissue carrier (Fig. 1 b, c). Pressing down a tension ring the biomaterial is fixed in position (Fig. 1 d, e). After mounting the tissue carrier is enveloped and sterilized for example in an autoclave. Subsequently the tissue carrier can be stored or used immediately for the intended culture protocol.

For cell seeding the mounted tissue carrier is placed by a forceps in a 24-well culture plate (Fig. 2a). To concentrate cells on the tissue carrier culture medium is added to a level so that the selected biomaterial is just wetted. Then cells are added within a small droplet of medium. Culture is started using conventional media inside a CO_2-incubator until adhesion becomes perfect. The kind and degree of cell adhesion can be registered by fluorescence labeling (Fig. 2b–e) [25]. An individual culture experiment shows propidiumiodide-labeled nuclei of MDCK cells grown in the same medium for 3 days on 4 different biomaterials such as glass (Fig. 2b), thermanox (Fig. 2c), white (Fig. 2d) and black (Fig. 2e) polycarbonate. Each of the specimens exhibits an individual growth pattern ranging between confluent attachment (Fig. 2b), dome (Fig. 2c), blister (Fig. 2d) and cluster (Fig. 2e) formation. This basic experiment shows the enormous influence of a selected biomaterial on the growth pattern of cells.

3. Tissue-specific adhesion

When cells are cultured in the static environment of a conventional dish, the side of cells resting on the bottom receives a significantly reduced supply of nutrients and oxygen (Fig. 3a). The situation is improved, when a tissue carrier is used, since cells are provided now from the upper and lower side by medium (Fig. 3b). This way a standard culture is started by seeding cells onto the upper side of the selected biomaterial. When a tissue carrier is turned, cells can be seeded also on the basal side (Fig. 3c). Thereby co-culture with two different cell types becomes possible (Fig. 3d) [26,27]. Furthermore, a slice of tissue can be mounted between two pieces of a woven net of fleeces (Fig. 3e) [28,29]. Flexible materials with adherent cells are clamped in a modified tissue carrier like the skin on a drum (Fig. 3f) [30,31]. Thus, a variety of biomaterials can be used in combination with cells on a mounted tissue carrier.

4. Compatible perfusion culture containers

The static environment within a culture dish leads to an uncontrollable increase of metabolites and a decrease of nutrition during time. To offer a constant provision with always fresh medium a tissue carrier with seeded cells is used in a perfusion container during long term culture. All of the containers are machined out of Makrolon® so that they can be autoclaved for multiple uses. The exact geometrical placement of the tissue carrier within a perfusion culture container guarantees a constant provision with fresh nutrition from all sides. One type of container allows the simple bathing of cells or tissue under continuous medium perfusion (Fig. 4a). In a gradient container the tissue carrier is placed between the base and lid so that the luminal and basal side can be provided with individual media mimicking a natural environment for epithelia (Fig. 4b). Another culture container is made of a transparent lid and base allowing the microscopic observation of the developing tissue (Fig. 4c). A special container exhibits a flexible silicone lid (Fig. 4d). Applying force to this lid by an eccentric rotor simulates a mechanical load as required in cartilage and bone regeneration. Shaped tissues such as an auricle or different forms of cartilage can be generated in a special tissue engineering container (Fig. 4e). Finally, spatial

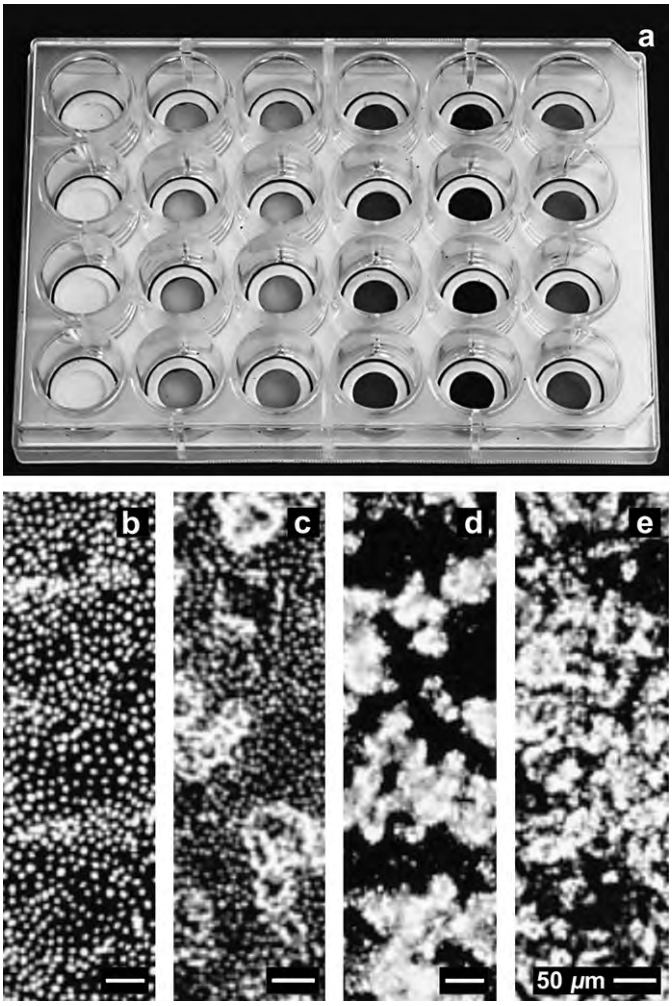


Fig. 2. (a) Tissue carriers including different biomaterials in a 24-well culture plate. Pattern of propidiumiodide-labeled MDCK cells grown for 3 days on 4 different biomaterials mounted in a tissue carrier. The individual growth pattern is ranging between (b) confluent attachment, (c) dome, (d) blister and (e) cluster formation.

development of tubules derived from renal stem/progenitor cells is promoted within a perfusion container at the interface of an artificial interstitium made of a polyester fleece (Fig. 4f) [28,29]. To maintain the desired temperature of 37 °C within a perfusion culture container a heating plate (MEDAX-Nagel, Kiel, Germany) and a cover lid (not shown) is used (Fig. 5).

5. Transport of culture medium

Permanent transport of culture medium is best accomplished using a slowly rotating peristaltic pump (ISMATEC, IPC N8, Wertheim, Germany), which is able to deliver adjustable pump rates of 0.1–5 ml per hour. During long term culture the growing tissue is always exposed to fresh medium, while the metabolized medium is collected in a separate waste bottle and is not re-circulated (Fig. 5). On the passage through the container the medium flows along an inserted tissue carrier. This way a growing tissue is continuously supplied with fresh medium preventing an unphysiological accumulation of metabolic products and an overshoot of paracrine factors. Thus, a natural exchange of fluid is simulated, molecules with high affinity are concentrated around the developing tissue, while factors with low affinity are eliminated by the effluent medium.

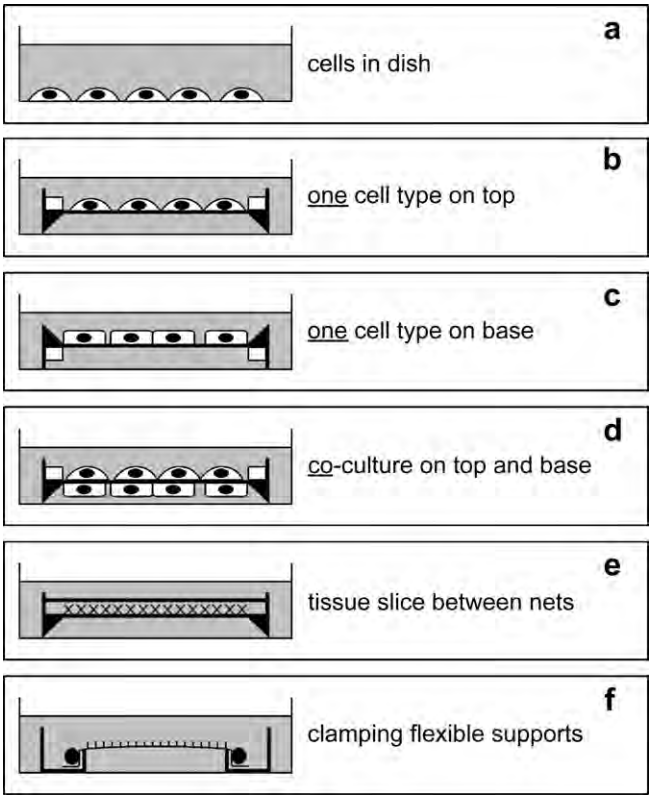


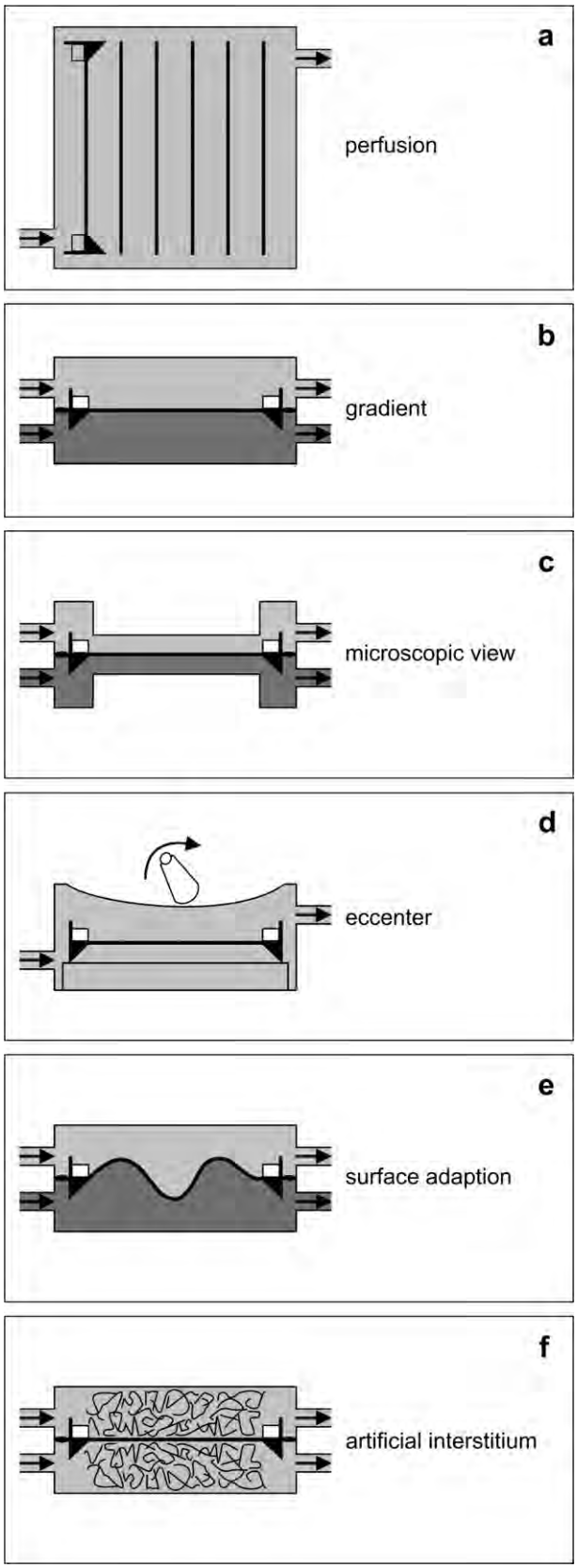
Fig. 3. Variations of cell seeding. (a) Cells resting on the bottom of a conventional dish receive a reduced supply of nutrients and oxygen. (b) On a tissue carrier cells are provided from the upper and lower side. (c) Turning a tissue carrier cells can be seeded also on the basal side. (d) Using two different cell types co-culture becomes possible by seeding cells on the upper and lower side. (e) A slice of tissue is mounted between two nets. (f) Flexible materials are fixed on a modified tissue carrier.

6. Keeping constant pH

Cultures in a CO_2-incubator are usually buffered by a system consisting of a relatively high amount of $NaHCO_3$ and a 5%-CO_2 atmosphere to maintain a constant pH of 7.4. If such a formulated medium is used for perfusion culture outside a CO_2-incubator, the pH shifts from the physiological range to alkaline values due to the low content of CO_2 (0.3%) in atmospheric air. For that reason any medium used outside a CO_2-incubator has to be stabilized by reducing the $NaHCO_3$-concentration and/or by adding biological buffers such as HEPES (GIBCO/Invitrogen, Karlsruhe, Germany) or BUFFER ALL (Sigma–Aldrich–Chemie, München, Germany). The necessary amount can be determined by admixing increasing amounts of biological buffer solution to an aliquot of medium and letting it equilibrate over night on a thermo plate under atmospheric air. For example, application of 50 mmol/l HEPES or an equivalent of BUFFER ALL (ca. 1%) to IMDM (Iscove's Modified Dulbecco's Medium, GIBCO/Invitrogen) will maintain a constant pH of 7.4 throughout long term perfusion culture under atmospheric air on a laboratory table.

7. Equilibration of oxygen

To obtain optimal equilibration of the pH, pO_2 and pCO_2 in a perfusion culture set-up, a medium such as IMDM is pumped through a gas-permeable silicone tube. This silicone tube provides a large surface for gas exchange by diffusion due to a thin wall (1 mm), the small inner diameter (1 mm) and its extended length



(1 m). Analysis of IMDM (3024 mg/l $NaHCO_3$, 50 mmol/l HEPES) equilibrated against atmospheric air in such a standard perfusion culture set-up shows partial pressures of 160 mmHg O_2 and 12 mmHg CO_2 [32].

8. Modulation of oxygen

Growing cells and tissues have individual oxygen requirements. For that reason it is important that the oxygen content can be adapted. The technical solution is a gas exchanger module with a gas inlet and outlet housing inside a spiral of long thin-walled silicon tube for medium to pass through (Fig. 5). Since the tubing is highly gas-permeable, it guarantees optimal diffusion of gases between culture medium and surrounding atmosphere during run. The desired gas atmosphere within the exchanger is maintained by a constant flow of a specific gas-mixture through the module. This way the content of oxygen or any other gases can be modulated in the medium by diffusion preventing infection by microorganisms. For example, using this simple protocol it became possible to decrease the oxygen partial pressure within the medium under absolutely sterile conditions [32].

9. Elimination of gas bubbles

During long term perfusion culture it is observed that gas bubbles form not only during suction of medium but also at material transitions. These microscopic gas bubbles are transported with the culture medium, increase in size and eventually form an embolus that massively impedes medium flow. Gas bubbles can also accumulate in the culture container leading to a regional shortage of medium supply, causing erratic breaks in the fluid continuum and resulting in massive pressure changes. In a gradient perfusion culture set-up, where two media have to be transported at exactly the same speed and pressure such effects can lead to pressure differences, which in turn destroy the growing tissue [33].

To avoid the concentration of gas bubbles within a perfusion culture container a gas expander module was developed that removes it from the medium (Fig. 5). When medium is entering the gas expander module, it rises within a small reservoir and expands before it drops down a barrier. During this process gas bubbles are separated from the medium and collected at the top of the gas expander module. Medium leaving the container is oxygen-saturated but bubble-free [33].

10. Tissue-specific applications of the perfusion culture technology

10.1. Epithelia

10.1.1. Development of epithelia in perfusion culture

Minusheet® tissue carriers and perfusion culture systems were developed to improve environmental factors affecting differentiation of renal derived embryonic cells [34,35]. Applying this new technique for the first time cell biological features could be raised as

Fig. 4. Use of a tissue carrier in different perfusion culture containers. (a) In a first type of perfusion culture container the tissue is provided with always fresh medium. (b) In a second type the tissue is exposed to a luminal/basal medium gradient. (c) In a microscope container with a transparent lid and base growing tissue can be observed. (d) A flexible lid made of silicone and a rotating eccenter expose the tissues to liquid pressure differences. (e) Tissues are shaped on a three-dimensional biomaterial during perfusion culture. (f) A polyester fleece is featuring an artificial interstitium in a perfusion container for the spatial development of tissue.

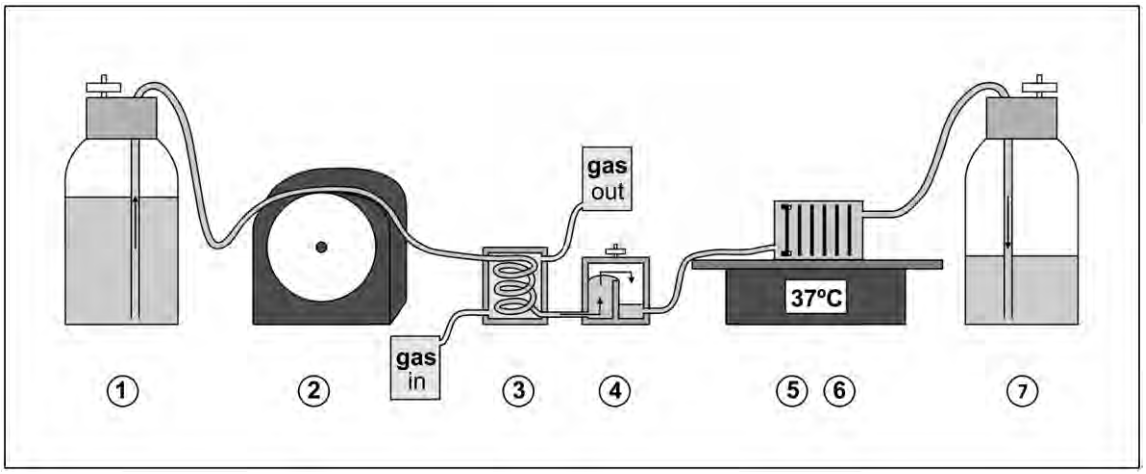


Fig. 5. Perfusion culture set-ups are self-contained and can be used on a laboratory table. A thermo plate and a Plexiglas lid (not shown) maintain the desired temperature. During culture a peristaltic pump transports the medium (1.25 ml/h) from the storage bottle on the left side to the waste bottle on the right side. Respiratory gases can be adapted by a gas exchange module containing a silicone tube. Gas bubbles in the transported culture medium are eliminated in a gas expander module before reaching the perfusion culture container. 1 – storage medium bottle, 2 – peristaltic pump, 3 – gas exchange, 4 – expander for gas bubble elimination, 5 – thermoplate, 6 – perfusion culture container, 7 – waste medium bottle.

found in adult Principal and Intercalated cells of the renal collecting duct [31,36–39]. Special attention was then directed to the selection of improved biomaterials supporting an optimal adhesion for cells in perfusion culture [25]. In consequence, these new experiences brought new insights in the spatial development of glomeruli and microvasculature made by perfusion culture [40–43].

Performing perfusion culture vessel formation [44,45] and regenerative activity of endothelium [46,47] could be tested under improved experimental conditions. To investigate living conservation for tissue engineering human gingival epithelium was generated in long term perfusion culture [48,49]. Factors affecting reproductive aging and the development of fertilized eggs were investigated with anterior pituitary gland [50], oviduct epithelium [51] and endometrium [52]. Optimal matrix coating and influences of continuous medium flow were elaborated for hepatocytes [53–56]. Regeneration of urothelium was investigated in combination with newly developed stent material [57]. Effects of new pharmaceuticals on ciliary beat frequency (CBF) were performed with differentiated nasal epithelium kept in perfusion culture [58].

10.1.2. Epithelia in gradient perfusion culture

During fetal development epithelia are exposed to the same medium at the luminal and basal side. In contrast, when the junctional complex in adult epithelia is sealing during differentiation different media are yet present at the luminal and basal side. In comparison, at the bottom of a conventional culture dish all sides of an epithelia cell are exposed to the same medium. This specific situation leads to a permanent short circuit promoting growth but suppressing polar differentiation [59–64]. To overcome this problem and to simulate a specific environment for epithelia Minusheet® gradient perfusion culture was developed.

10.1.3. Renal collecting duct epithelium

Experiments with gradient perfusion culture were started to investigate the influence of different fluids at the luminal and basal side of an embryonic renal collecting duct (CD) epithelium [30,31,35,65–71]. It was found that the process of development in a CD epithelium has an unexpected long latent period of 3 days and takes at least 10 days until differentiation is completed. The development is dependent on increasing NaCl concentrations offered at the luminal side (Fig. 6). Only in a continuous electrolyte gradient over days specific features such as binding for PNA-lectin and site-specific monoclonal antibodies were found to be up-regulated. However, when high NaCl content at the luminal side was replaced against low NaCl content specific features were down-regulated. Thus, gradient perfusion culture made it for the first time feasible to investigate the development of an embryonic renal epithelium under realistic conditions. In addition, learning from the tissue-specific development of epithelia the used media were adapted to the individual physiological requirements in further successfully performed perfusion culture experiments [32,72,73].

10.1.4. Retina

Retina is a complex tissue lined by a pigment epithelium that cannot be maintained in the static environment of a culture dish over prolonged periods of time. For that reason intact retina was mounted in a tissue carrier and cultured in a gradient perfusion container [74–80]. It was demonstrated that neurons and pigment epithelium maintain a perfect morphology for at least 10 days. This experimental outcome illuminates new perspectives for safety testing of pharmaceuticals designed for intraocular application and shows challenging experimental options in the wide field of retina degeneration, damage and repair.

10.1.5. Blood–retina and blood–brain barrier tests

Blood–retina and blood–brain barrier are crucial for the transport of pharmaceuticals. To simulate blood–retina and blood–brain barrier under realistic in vitro conditions gradient perfusion culture is an ideal technique [81,82]. The experimental set-up exhibited new permeation features and displayed an intact polarized expression of efflux pumps such as multidrug resistance protein (P-gp) and multidrug resistance-associated protein (MRP).

10.1.6. Blood–air barrier

A specific environment for pneumocytes at the blood–air barrier was simulated in gradient perfusion culture [26]. When pneumocytes and endothelial cells were co-cultured on a polycarbonate filter within a gradient perfusion container a junctional complex was found to develop sealing tightly the blood–air barrier. In addition, characteristic features of polar differentiation within the epithelia were up-regulated. It was concluded that gradient perfusion culture in combination with pneumocytes and endothelial cells is a realistic model to investigate dose controlled exposure of airborne particles. Moreover, features of a dose

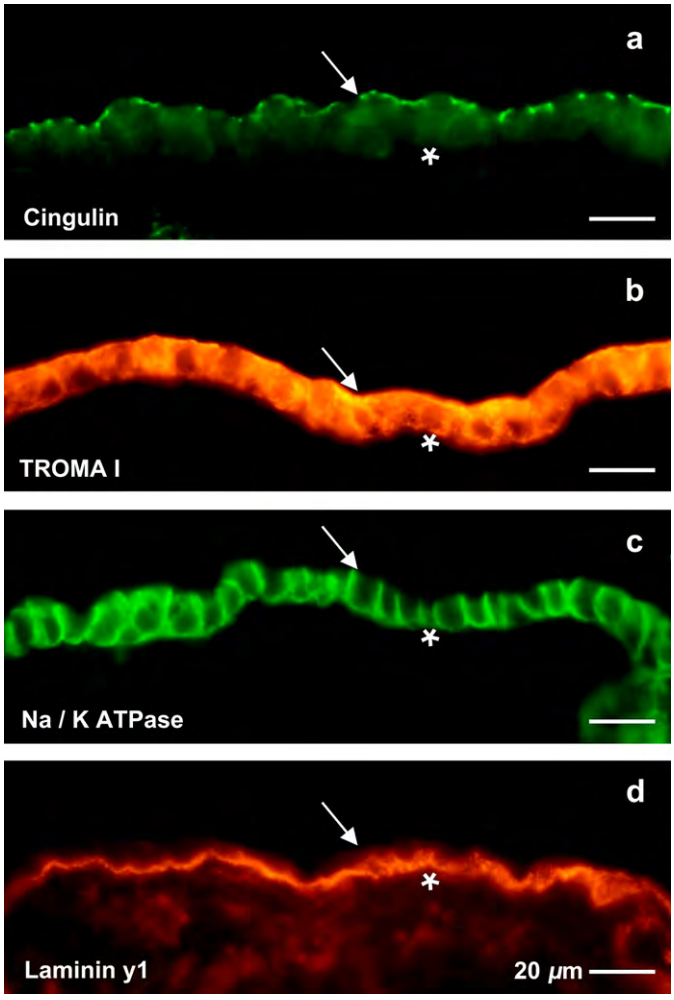


Fig. 6. Cell biological features of an embryonic renal collecting duct (CD) epithelium kept for 13 days in a gradient luminal: IMDM + aldosterone (1×10^{-7} M) + 15 mmol/l NaCl/basal: IMDM + aldosterone (1×10^{-7} M). Immunohistochemistry shows a positive label for (a) cingulin, (b) TROMA I, (c) Na/K ATPase and (d) laminin y1. In contrast, perfusion culture of epithelia with same media at the luminal and basal side do not develop these features. Basal lamina (asterisk), lumen (arrow).

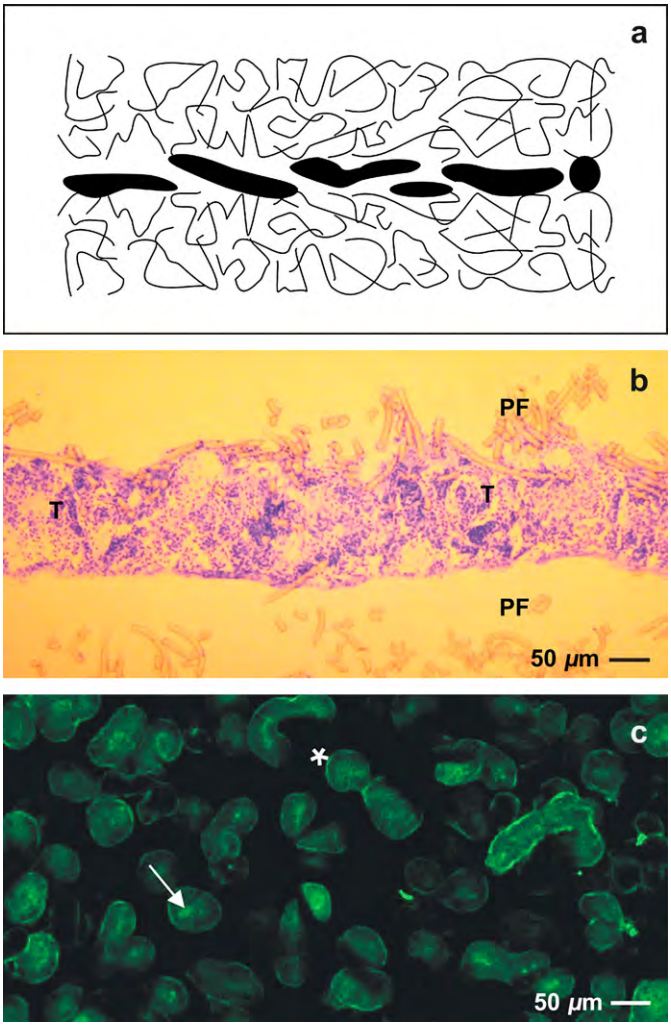


Fig. 7. Generation of renal tubules at the interface of an artificial interstitium. (a) Schematic illustration shows that numerous tubules (T) develop between layers of a polyester fleece (PF). (b) Toluidin blue stained cryosection of generated tubules. (c) Soybean agglutinin (SBA)-labeled whole mount specimen of generated tubules. The tubules exhibit a basal lamina (asterisk) and a lumen (arrow).

controlled air–liquid interface (ALI) were investigated with A549 cells to elucidate barrier transport and repair mechanisms after alveolar injury [83–85].

10.1.7. Blood–gas barrier

Experiments on fish swim bladder gas gland were successfully performed in a gradient perfusion culture container [86]. In this case cells of gas gland were cultured on a filter at the interface between gas and fluid medium. The epithelium revealed a typical polarity and functionality as observed in the environment of swim bladder gas gland in fish.

10.1.8. Pharmaceutical application

In the organism most of the administered pharmaceuticals have to pass an epithelial barrier. To test the transport of drugs through an epithelial cell layer long term gradient perfusion culture was performed [87,88]. It was further detected that gradient perfusion culture with Caco-2 cells was leading to tightly sealing epithelia [89,90]. Gradient perfusion culture revealed reproducible results much earlier than observed in traditional 21-day static cultures. The permeability coefficient of several model pharmaceuticals across Caco-2 cells was approximately twofold higher than obtained under static conditions.

10.1.9. Renewal of skin

Renewal of skin is an important research area in actual biomedicine. Thus, epidermis equivalents were generated using gradient perfusion culture [91]. Composite grafts of INTEGRA® matrix and human keratinocytes were cultured in a gradient container in order to evaluate better the potential for the cost-effective engineering of full-thickness skin grafts and the treatment of ulcers.

10.1.10. Regenerating vessels

Development on micro-vessels was investigated within a gradient perfusion container [44,45]. In these experiments the spatial generation of capillary-like structures was obtained. It was further shown that perfusion of medium in pulses promotes much better the development of a capillary-like network than a continuous transport.

10.1.11. Non-epithelial barrier

Beside the epithelial cell layers also other tissues exhibit important barrier functions. Experiments related to such non-epithelial

barriers were performed with dentin discs in a gradient perfusion container [92–100]. Actual data show that polymerized dental resin materials release residual monomers that may interact with pulp tissue. In consequence, this modified dentin barrier test revealed as an ideal model to investigate long term toxic effects of new biomaterials under realistic in vitro conditions. Furtheron, new insights in permeability testing and degradation of gelatin membranes were obtained by using fibroblasts in a gradient perfusion container [101].

10.1.12. Generation of tubules at the interface of an artificial interstitium

To investigate the spatial development of renal tubules, an advanced perfusion culture set-up was developed (Figs. 4f and 7a). In these experiments renal stem/progenitor cells were mounted between two layers of polyester fleece. The interface between them serves as an artificial interstitium [69,102,103]. Numerous experiments demonstrate that the culture at the interface of an artificial interstitium is an effective technique to investigate the process of tubule regeneration (Fig. 7b) [104,105]. Surprisingly, the process of tubule formation is induced by aldosterone [105–107]. Since antagonists such as spironolactone or canrenoate prevent tubulogenic development, the hormonal signal is mediated via the mineralocorticoid receptor (MR) [108,109]. Disturbing the molecular interaction between MR and heat shock protein 90 by geldanamycin results in a lack of tubule formation.

The interface between two layers of polyester fleece promotes the spatial development of numerous tubules [108–110]. Using an artificial interstitium the surrounding of generating tubules is not stacked by a coat of extracellular matrix proteins. Thus, for the first time it was possible to investigate the synthesis of interstitial molecules such as collagen type III during generation of tubules [111]. Actual data show that generating tubules avoid a direct contact to each other by keeping a minimal distance during spatial development (Fig. 7c). The separation is caused by a link between the basal lamina of tubules and newly synthesized fibers of the extracellular matrix. Recent experiments show that different kinds of polyester fleeces reveal challenging options for regenerating tubules in biomedicine [29].

11. Connective tissue

A great issue in regenerative medicine is the treatment of cartilage and bone defects by artificial tissue constructs containing various scaffold materials. In numerous cases perfusion culture could improve the quality of generated tissues.

11.1. Cartilage

To generate cartilage constructs for implantation bioresorbable scaffolds were frequently applied in combination with chondroblasts/-cytes within a culture dish. However, in the static environment an increasing concentration of biodegraded molecules such as lactate is liberated resulting in a damage of the growing tissue during time. In consequence, to eliminate continuously biodegraded molecules Minusheet® perfusion culture was applied successfully for the generation of cartilage [18,19,112,113]. Applying this method it became possible to elaborate realistic date concerning kinetics of the degradation process from different scaffold materials [114,115]. In addition, by perfusion culture the cell biological quality of generated tissue could be improved by stepwise modifications of the scaffold material so that the risk for implantation could be minimized [116–122]. Surprisingly, it was shown that the application of natural extracellular matrix such as a conventional collagen sponge does not improve the quality of generated cartilage [123]. In contrast, scaffold materials with modified polyethylene coating [124] or gelatine-based Spongostan [21] focused in excellent results. Further on, the generation of intact cartilage constructs by perfusion culture revealed as an ideal model to investigate factors affecting destructive joint diseases [125–127].

11.2. Bone

In bone tissue engineering described perfusion culture technique was applied to investigate the development of osteoblasts on ceramic materials [22–24], decellularized spongeous bone [128], collagen membranes [129], mineralized collagen [130,131], hydroxyapatite scaffold [132,133], PLGA sheets [134], laminin-coated polycarbonate membranes [27] and textile chitosan scaffolds [135,136]. Most important for clinical applications are experiments related to effects influencing tissue development after sterilization of scaffold materials consisting of poly-D,L-lactic-*co*-glycolic acid [134]. A further problem is the occurrence of unstirred layers of fluid within a tissue engineered construct, since it develops during perfusion culture a permanently increasing thickness. For that reason a constant provision with nutrition and oxygen plays an essential role during generation of the construct [137]. Last but not least, learning from bone in perfusion culture may lead to an effective strategy for the regeneration of dentin [96].

12. Muscular tissue

Only two papers were found dealing with the generation of muscular tissue in perfusion culture. During culture of gastric mucosa it was observed that smooth muscular tissue is developing in the lamina propria [64]. Using an improved biomaterial for cell anchorage numerous cerebral pericytes were found to express site-specific pericytic aminopeptidase N/pAPN [138].

13. Nervous tissue

A central theme in neurology is the escape of dopamine synthesis during Parkinson disease. To investigate expression of dopamine Minusheet® perfusion culture was performed successfully with mesencephalic neurons [139]. In this coherence it was found that neurothrophins stimulate the release of dopamine via Trk and p75Lntr receptors. Further it was shown that in hippocampal neurons and the pheochromacytoma cell line PC 12 application of exogenous neurotrophins exerts positive feedback effects on secretion of synthesized neurotrophins. This pathway is mediated via an activation of tyrosine kinase neurotrophin receptors [140]. An important role plays the influence of sodium in an activity-dependent secretion of neurotrophins [141]. Furthermore, differences in the secretion between nerve growth factor and brain-derived neurotrophic factor were observed [142]. Finally, SH-SY5Y human neuroblastoma cells were found to differentiate into a neuronal-like state in long term perfusion culture [143]. The cells could be kept in an active state for more than 2 month without the need of passage. In other coherence RAT-1 fibroblasts were investigated expressing Cypridina noctiluca luciferase driven by the promoter of a circadian clock gene Mma11 [144]. In so far the CLuc reporter assay in combination with described perfusion culture appears as an innovative pharmacological tool for drug discovery.

14. Conclusions

This paper has described a modular system to improve the environment of cultured cells and tissues used in areas such as tissue engineering and microreactor technology; a fuller analysis of the literature on this Minusheet® perfusion culture technology is listed: www.biologie.uni-regensburg.de/Anatomie/Minuth/proceedings.htm.

The technical concept is based on individually selected biomaterials, tissue carriers and different kinds of compatible perfusion culture containers. During long term culture the growing tissue is continuously exposed to fresh medium.

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