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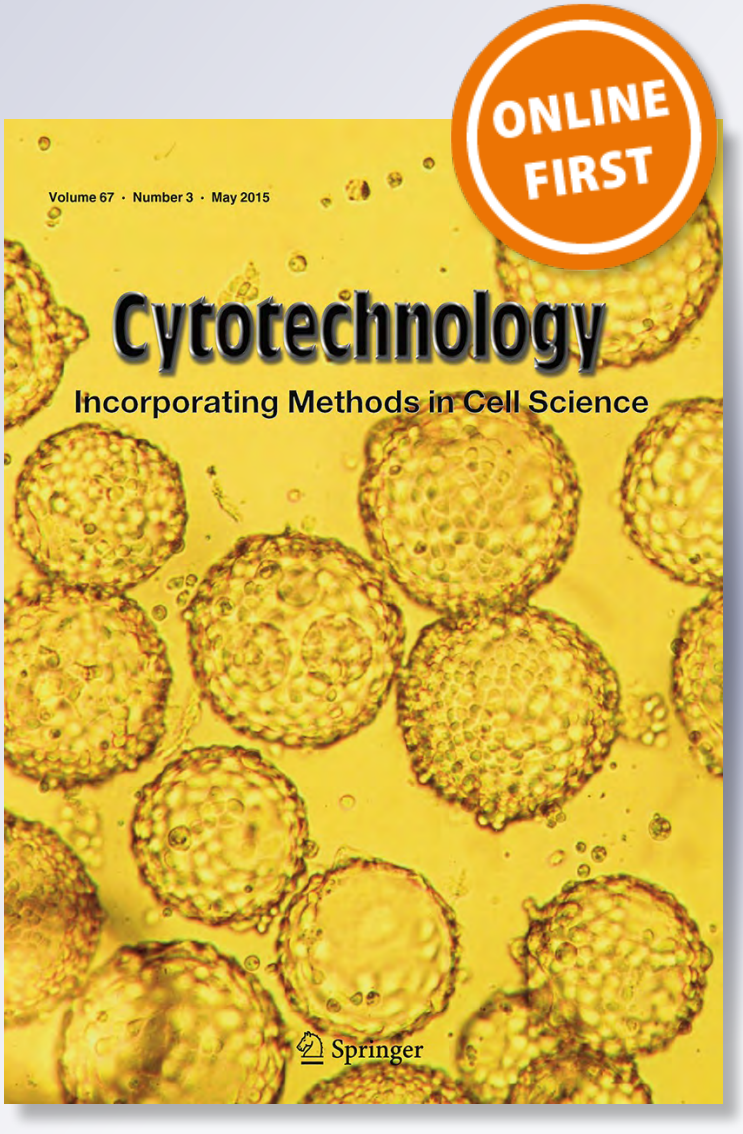

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REVIEW

Bridging the gap between traditional cell cultures and bioreactors applied in regenerative medicine: practical experiences with the MINUSHEET perfusion culture system

Will W. Minuth · Lucia Denk

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Abstract To meet specific requirements of developing tissues urgently needed in tissue engineering, biomaterial research and drug toxicity testing, a versatile perfusion culture system was developed. First an individual biomaterial is selected and then mounted in a MINUSHEET® tissue carrier. After sterilization the assembly is transferred by fine forceps to a 24 well culture plate for seeding cells or mounting tissue on it. To support spatial (3D) development a carrier can be placed in various types of perfusion culture containers. In the basic version a constant flow of culture medium provides contained tissue with always fresh nutrition and respiratory gas. For example, epithelia can be transferred to a gradient container, where they are exposed to different fluids at the luminal and basal side. To observe development of tissue under the microscope, in a different type of container a transparent lid and base are integrated. Finally, stem/progenitor cells are incubated in a container filled by an artificial interstitium to support spatial development. In the past years the described system was applied in numerous own and external investigations. To present an actual overview of resulting experimental data, the present paper was written.

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Keywords Cell culture · Perfusion culture · 3D culture · Tissue carrier · Bioreactor · Tissue engineering · Biomaterial testing · Biomedicine

Introduction

Nowadays it is standard in the laboratory to culture cells and tissues according to their individual needs by more or less sophisticated techniques. However, 25 years ago proliferating cells were generally kept in a small selection of glass or plastic containers resembling the traditional Petri dish. The problem was that a dish does not meet the requirements of developing tissues. Thus, for the generation of tissues improved culture techniques were needed but at that time attractive bioreactors were not commercially available.

As a consequence, the lack of suitable tools for the generation of specialized tissues was the motivation to start with the construction of the MINUSHEET® perfusion culture system (Minuth 1990). The goal was to devise a simple technique, which enables selection of an individual biomaterial for optimal cell adhesion to mount it in a specific holder, to seed cells on it in a 24 well culture plate and finally to transfer it to a series of perfusion culture containers for the generation and long term maintenance of various specialized tissues (Minuth and Rudolph 1990; Minuth et al. 1992a). Considering further the diversity of specialized tissues in an organism on the

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one hand and the variety of biomaterials used in tissue engineering and regenerative medicine on the other hand, it was obvious that only a highly adaptable system could provide those environmental parameters that are demanded for corresponding in vitro experiments.

Technical properties

Mounting a biomaterial in a tissue carrier

The introduced concept is based on a MINUSHEET® tissue carrier, which enables the user to mount a selected biomaterial by his own hands in the laboratory. To stay compatible with a 24 well culture plate, the biomaterial is punched out to a diameter of 13 mm (Fig. 1a). In this coherence it does not matter whether decellularized extracellular matrix, synthetic polymers, ceramics, metals or biodegradable scaffolds are selected. Further on, materials can be used in form of foils, filters, nets, fleeces, foams or solid supports containing small or big pores.

For practical application, a punched out filter is placed in the base part of a MINUSHEET® tissue carrier (Fig. 1b; black ring). By pressing down a tension ring (white ring) the filter is fixed in position. The use of this demonstrated tissue carrier prevents damage of the mounted biomaterial and protects cells during seeding, ongoing development and further experimental manipulation.

The following disinfection of the mounted carrier depends on the chemical composition of the selected biomaterial. Therefore it is either performed by formaline, ethylene oxide gas, irradiation or autoclaving. Subsequently, the tissue carrier can be frozen, stored at room temperature in a sterile box or used immediately for cell seeding.

Seeding of cells

For seeding of cells a sterile tissue carrier mounted with a biomaterial is placed by forceps into a 24 well culture plate (Fig. 1c). In a next step culture medium is slowly added by a pipette so that the surface of the inserted biomaterial is just wetted. Then cells are transferred by a pipette within a small droplet of medium. In a standard set up seeding of cells is performed only on the upper side of a selected biomaterial. However, in the case a co-culture experiment is planned, seeding of a second cell type is made after turning the tissue carrier.

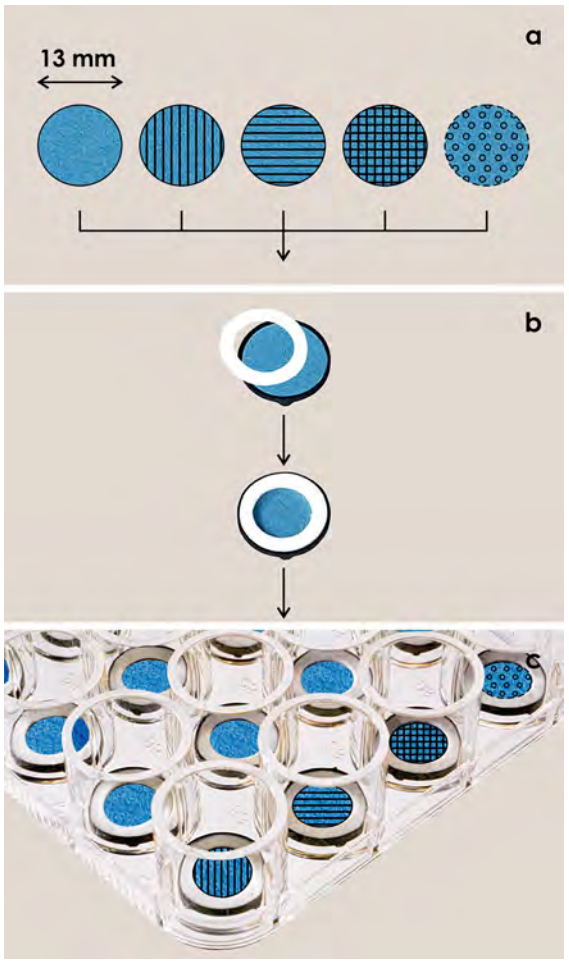


Fig. 1 Application of a MINUSHEET® tissue carrier. **a** First a biomaterial measuring 13 mm in diameter is selected. **b** Then the biomaterial is placed in the black base part of a tissue carrier. Mounting is completed by pressing the white tension ring in the base part. **c** After sterilization the carrier is transferred by forceps to a 24 well culture plate for cell seeding

For creation of an artificial interstitium isolated cells or a thin slice of living tissue are mounted between two pieces of polyester fleece in a carrier. Further pieces of a collagen sheet can be placed in a tissue carrier like the skin of a drum. These few examples illustrate that in principle numerous kinds of applications exist for mounting a biomaterial in combination with isolated cells or even living tissues in a carrier.

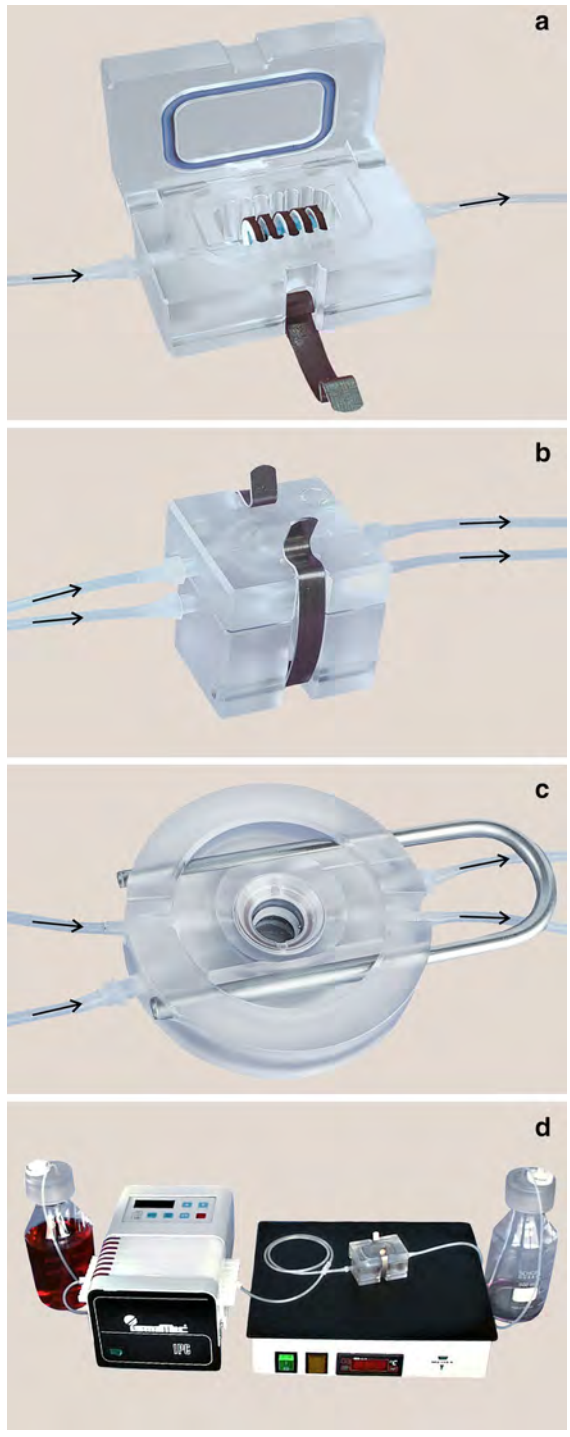


Fig. 2 Versatile use of a MINUSHEET® tissue carrier in a perfusion culture container. **a** In a basic version a perfusion culture container can hold six tissue carriers for provision with always fresh medium. **b** In a gradient perfusion culture container the contained tissue is exposed to different fluids at the luminal and basal side. **c** For observation of growing tissue under a microscope a transparent lid and base is integrated in a container. **d** A perfusion culture set up is running in the typical case on a laboratory table and under atmospheric air. A thermo plate maintains the desired temperature of 37 °C. During culture a peristaltic pump transports the medium (1.25 ml/h) from a storage bottle (*left side*) to the waste bottle (*right side*). *Arrow* indicates flow of medium

Analysis of cell distribution

Regardless of whether transparent or non-transparent biomaterials are used, the quality of seeding and resulting cell distribution must be controlled. This can best be achieved by epi-fluorescence microscopy, when specimens were fixed in 70 % ethanol and labeled for example by propidium iodide (Minuth et al. 1994). In such a protocol fluorescent nuclei reflect the distribution of cells on a selected biomaterial. Surprising results were obtained, when MDCK cells were cultured on materials such as glass, polystyrene (Thermanox®), white and black polycarbonate filters. Depending on the selected material the pattern of screened cells was ranging between perfect confluence, atypical dome, cysts and cluster formation.

Selection of a perfusion culture container

Only for the relatively short period of cell seeding a MINUSHEET® tissue carrier is kept in the static environment of a 24 well culture plate. Then a perfusion culture container is selected to offer adherent cells a fluid milieu, which better meets special needs of developing tissue than the static environment of a dish. Further the exact adjustment of a tissue carrier within a perfusion culture container guarantees an equal distribution and consequently a continuous transport of always fresh culture medium, whereby an uncontrollable accumulation of harmful metabolites and an overshoot of paracrine factors during proceeding culture is prevented.

In the basic version of a perfusion culture container up to six tissue carriers can be placed beside each other (Fig. 2a). A continuous fluid flow provides here for example developing connective tissue from all sides with always fresh nutrition and respiratory gas. In a gradient perfusion culture container the tissue carrier is fixed centrally between the base and the lid. This design enables to transport different media at the luminal and basal side (Fig. 2b). For microscopic observation a

container exhibits a transparent lid and base (Fig. 2c). A further container contains a flexible silicone membrane. When mechanical force by an eccentric rotor is applied, a transmitted stimulus in the interior supports development of cartilage or bone. Finally, a perfusion culture container filled with a polyester fleece as an artificial interstitium makes it possible to investigate spatial development of parenchyma.

Fresh fluid continuum

When cells in combination with a biodegradable biomaterial are kept in culture, it must be considered that both can produce harmful metabolites such as lactic acid leading in turn to an un-physiological accumulation. Thus, an overshoot of such metabolites must be prevented and its concentration has to be kept on a constant low physiological level. Due to this reason developing tissue is exposed in a perfusion culture container to a continuous flow of always fresh medium. To prevent an accumulation of harmful metabolites within a perfusion culture container, quality of medium is measured at the outflow for example by a blood gas analyzer (Nova Biomedical, Rödermark, Germany). According to registered metabolites the rate of medium transport can be adapted to the individual needs of contained tissue. Finally, metabolized medium is not re-circulated but collected in a separate waste bottle.

The transport of culture medium is best accomplished by application of a slowly rotating peristaltic pump (ISMATEC, IPC N8, Wertheim, Germany), which is able to provide adjustable transport rates between 0.1 and 5 ml per hour and channel (Fig. 2d). In personal experiments optimal results were obtained, when medium was transported with 1.25 ml/h for a period of at least 13 days. Further on, for maintaining a defined temperature of 37 °C within a perfusion culture container, a heating plate (MEDAX-Nagel, Kiel, Germany) and a special Plexiglas cover lid (not shown) is used (Fig. 2d).

Stabilization of pH in transported culture medium

Perfusion culture can be performed either in a traditional CO_2 incubator or better on a laboratory table. In the case a CO_2 incubator is used, a culture medium is selected containing a buffer system with a relatively high amount of $NaHCO_3$. It will maintain in a 5 % CO_2 atmosphere of an incubator a constant pH between 7.2 and 7.4. However, when such a formulated medium is used in a perfusion culture set up outside a CO_2 incubator, the pH will shift from the physiological range to alkaline values due to the low content of CO_2 (0.03 %) in atmospheric air. In turn contained cells, respectively, tissues are chronically damaged and will finally die.

In principle, most of media are suitable for application in perfusion culture. However, when it is performed outside a CO_2 incubator, the media must be ordered with a strongly reduced $NaHCO_3$ concentration. Further biological buffers such as HEPES (GIBCO/Invitrogen, Karlsruhe, Germany) or BUFFER ALL (Sigma-Aldrich-Chemie, München, Germany) have to be added for constant stabilization of pH. The necessary amount is determined by admixing increasing concentrations of biological buffer solution (always in the same volume) to an aliquot of medium. Then the medium must equilibrate overnight on a thermo plate at 37 °C under atmospheric air. Finally, the aliquots are measured by an electrolyte analyzer. The data revealed that for example addition of 50 mmol/l HEPES or an equivalent of BUFFER ALL (1 %) to IMDM (Iscove's Modified Dulbecco's Medium, GIBCO/Invitrogen) maintains the pH between 7.3 and 7.4 throughout long term perfusion culture on a laboratory table under atmospheric air (Roessger et al. 2009).

Further on, beside conventional media well suited culture media for perfusion culture running under atmospheric air are Leibovitz's L-15 Medium and CO_2 Independent Medium. Both were successfully applied under chemically defined conditions (Minuth et al. 2013; Minuth and Denk 2013).

Respiratory gas in transported medium

For enrichment of oxygen (O_2) in a perfusion culture set up medium is pumped through a gas-permeable silicone tube. It provides a large surface for the gas exchange by diffusion due to a thin wall (1 mm), small inner diameter (1 mm) and extended length (1 m). For example, IMDM (3024 mg/l $NaHCO_3$, 50 mmol/l HEPES) equilibrated against atmospheric air reveals in a standard perfusion culture set up partial pressures of 160 mmHg O_2 and 10 mmHg CO_2 (Minuth et al. 2001; Strehl et al. 2004).

Further the requirement for oxygen depends on specialization of the individual tissue. For that reason

in special cases the concentration of O_2 must be adapted in transported medium. A simple technical solution is a gas exchange module containing a gas inlet and outlet (Strehl et al. 2004). Further a spiral with a long thin-walled silicone tube for medium transport is mounted inside the module. Since the tube of the spiral is gas-permeable, diffusion of gases between culture medium (inside the spiral) and a given atmosphere (outside the spiral) within the gas exchange module takes place during transport. Applying this simple method the gas atmosphere can be adjusted by a constant flow of a specific gas mixture at the outside of the spiral. During run of such an experiment the content of gas at the in- and outflow of a perfusion culture container is controlled by a blood gas analyzer.

Elimination of gas bubbles

During transport of medium gas bubbles will arise in the perfusion culture set up. Problematic is that they impede the flow of medium. Surprisingly, formation of gas bubbles is observed during suction of medium from the storage bottle, during transport at material transitions between tubes and connectors, at the surface of developing tissue and at the outflow of a perfusion culture container. First gas bubbles are small so that they are not visible to the naked eye. However, during transport of medium they increase in size, fuse with each other and form an embolus that can massively impede medium flow. When gas bubbles accumulate inside a perfusion culture container, they cause a regional shortage of medium supply. Finally, formation of gas bubbles in a gradient perfusion container is leading to remarkable fluid pressure changes, although normally two media must be transported at exactly the same speed and pressure (Fig. 2b). Thus, an embolic effect caused by gas bubbles in one of the channels leads to massive pressure differences destroying in turn the barrier function of an interposed epithelium.

To minimize arise of bubbles in a culture set up, a gas expander module is placed before medium is entering the perfusion culture container (Minuth et al. 2004a, b). Inside a gas expander module medium is rising within a small reservoir and expands before it drops down after a barrier. During this process gas bubbles are separated from the medium and collected at the top of the gas expander module. As a result, culture medium leaving the gas expander module stays oxygen-saturated but is free of gas bubbles.

Design and construction

Described MINUSHEET® tissue carriers (Fig. 1) and perfusion culture containers (Fig. 2) were not designed for one-off application but for multiple use. Since the tools are exposed to numerous cycles of cleaning and sterilization during years, a special design had to be made and stringent requirements on material quality were necessary. To prevent unwanted cracks and alterations in material surface, tissue carriers were finally produced by injection molding with Pocan® thermoplastic polyester resin. The illustrated perfusion culture containers and related equipment such as gas expander and gas exchange modules were produced in a certified workshop by a computerized numerical controled (CNC) milling machine out of Makrolon® polycarbonate.

Featuring development of epithelia

In previous personal experiments it was observed that epithelial cells do spread in a dish very well but often they do not develop expected cell biological features. To support differentiation, environment for epithelia was improved by offering an individual extracellular matrix or biomaterial for adhesion and by provision with always fresh culture medium.

For example, collecting duct (CD) tubule cells derived from the embryonic parenchyma of neonatal kidney were isolated with the associated organ capsule and mounted in a MINUSHEET® tissue carrier. For the first time could be observed that these cells develop during subsequent perfusion culture into a polarized epithelium. Immunohistochemistry further demonstrated that harvested epithelia express the same cell biological features as observed in adult Principal (P) and Intercalated Cells (IC) of the collecting duct tubule (Herter et al. 1993; Minuth et al. 1993; Aigner et al. 1994, 1995).

Moreover, perfusion culture experiments gave new insights in the spatial development as well of renal microvasculature and glomeruli (Kloth et al. 1994, 1995, 1998a; Kloth and Suter-Crazzolara 2000) as even of intact gastric glands (Kloth et al. 1998b). In other experiments it was investigated to what extent regeneration can be influenced by engineered microvessels (Frerich et al. 2006, 2008) or isolated endothelial cells (Bakowsky et al. 2005; Hayashi et al.

2009). To evaluate perspectives of living conservation, human gingival epithelium was kept in long term perfusion culture (Lehmann et al. 1997; Lauer 2009). Co-culture of human oral keratinocytes with osteoblast-like cells gave new insights for performance of hard and soft tissue reconstruction in future (Glaum et al. 2010).

Factors influencing reproductive aging and the development of fertilized eggs were screened with anterior pituitary gland cells (Zheng et al. 2007), oviduct epithelium (Reischl et al. 1999) and endometrial cells (Tiemann et al. 2005). New protocols for an optimal matrix coating and adaptation to continuous medium flow were elaborated for hepatocytes (Fiegel et al. 2004; Schumacher et al. 2007; Du et al. 2008; Xia et al. 2009). Regeneration of urothelium was analyzed in combination with newly developed stent materials (Sternberg et al. 2004). Finally, effects of newly developed drugs on ciliary beat frequency (CBF) were elaborated with the help of nasal epithelium kept in perfusion culture (Dimova et al. 2005). Reconstruction of cornea became possible by modulation of environment under dynamic culture conditions (Wu et al. 2014). Finally, reactions of retinal pigment epithelium kept in perfusion culture could be registered after laser irradiation by two-photon microscopy (Miura et al. 2013).

Renal epithelia exposed to a gradient

Past and present experiments revealed that gradient perfusion culture answers unsolved questions in developmental biomedicine. During the embryonic and early fetal period epithelia are still exposed to the same fluid at the luminal and basal sides due to still leaky barrier characteristics. However, in maturing epithelia a tight junction complex and up-regulated transport features form a functional barrier. To investigate such processes, a MINUSHEET® tissue carrier with epithelial cells seeded on different biomaterials was mounted in a gradient perfusion culture container (Fig. 2b). Transportation of different fluids through the lid and base part of the container produces a specific environment for epithelia. When this strategy was followed, for example intact renal barriers could be generated (Dankers et al. 2010, 2011).

Application of a gradient perfusion culture container made it further possible to investigate the influence on differentiation of different fluid composition at the luminal and basal sides of embryonic renal collecting duct (CD) epithelia (Minuth et al. 1992b, 1997a, b, 1999, 2001, 2005a; Steiner et al. 1997, Schumacher et al. 2002a; Minuth et al. 2009a). In the course of performed experiments it was detected that development of a CD epithelium starts with an unexpected long latent period of three days and needs at least 10 days for up-regulation of typical signs of differentiation. Further on, development can be triggered by increasing concentrations of NaCl administered at the luminal side. In such an electrolyte gradient over days typical epithelial cell characteristics such as TROMA I (Cytokeratin Endo-A; Fig. 3a), cingulin (Fig. 3b) or Na/K ATPase α5 (Fig. 3c) were up-regulated. Most interestingly, when fluid with an increased NaCl concentration at the luminal side was replaced against a low NaCl concentration, achieved characteristics were down-regulated within few days. This result illustrates that a luminal-basal electrolyte gradient maintains functional features within renal epithelia.

Challenging experiments were performed with hydrogel mounted in a MINUSHEET® tissue carrier

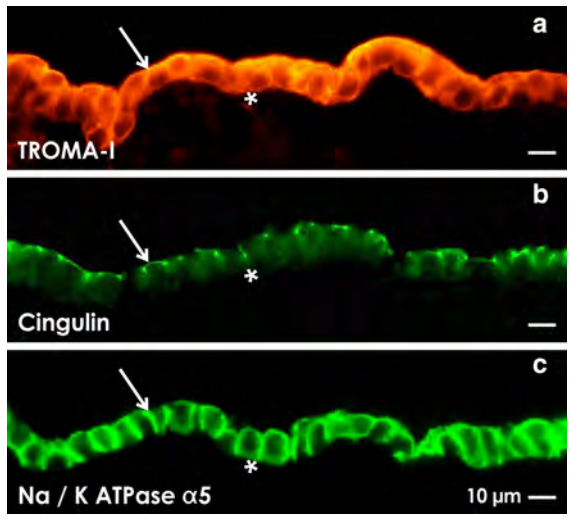


Fig. 3 Features of a renal collecting duct (CD) epithelium kept for 13 days in a gradient perfusion culture container. At the luminal side IMDM + aldosterone (1×10^{-7} M) + 15 mmol/l NaCl, while at the basal side IMDM + aldosterone (1×10^{-7} M) was transported. Immunohistochemistry shows that an intense label for tissue-specific markers such as **a** TROMA I, **b** cingulin and **c** Na/K ATPase α5 is present. Site of the basal lamina is marked by an *asterisk*, while lumen is indicated by an *arrow*

to substitute the glomerular basement membrane. In this experimental set up endothelial cells are seeded on the one side, while podocytes were growing on the other side. When those co-cultures were mounted in a gradient perfusion container, development of an intact urine-blood barrier has taken place so that related functions can be tested under advanced culture conditions (Bruggeman et al. 2012). Further on, testing of special bilayered scaffolds with tailorable properties in perfusion culture helps to optimize long term adherence and special differentiation of renal epithelial cells (Mollet et al. 2014).

Finally, epithelia kept in gradient perfusion culture illustrated that commercially available media often do not contain all of the compounds normally needed for optimal cell differentiation. As a consequence, for special demands culture media have to be adapted by addition of defined electrolyte concentrations so that an adequate degree of differentiation is achieved (Schumacher et al. 1999, 2002b).

Pigment epithelium in combination with retina

Retina is a complex neural cell composition that is delimited by a pigment epithelium. Since typical morphological features cannot be maintained in static environment of a dish, intact retina was mounted in a tissue carrier for perfusion culture in a gradient container (Framme et al. 2002; Spiegel et al. 2002; Saikia et al. 2006; Jian and Jingbo 2007; Hamilton et al. 2007; Hammer et al. 2008; Kobuch et al. 2008). These experiments illustrated that the pigment epithelium and neighboring neurons maintain a perfect morphology for a culture period of at least 10 days. On the one hand these exciting findings illustrate novel perspectives for safety testing of newly developed pharmaceuticals designed for intraocular application. On the other hand these experiments give rise to new opportunities for investigating the wide field of retina inflammation, aging, degeneration and repair by the help of an adequate culture system (Klettner and Roider 2009, 2012; Klettner et al. 2009; Miura et al. 2010; Treumer et al. 2012). In this coherence, for Example, molecular regulation of vascular endothelial growth factor secretion and cell biological reactions after fucoidan exposure were investigated (Klettner et al. 2013, 2014; Dithmer et al. 2014).

Blood-retina and blood–brain barrier

Blood-retina and blood–brain barriers are of special interest for the transport of new medicines. It has been shown that a MINUSHEET® tissue carrier in combination with a gradient perfusion container is an ideal tool to elaborate special features of these barrier functions under in vitro conditions closely adapted to nature (Steuer et al. 2004, 2005; Hamilton and Leach 2011). In turn, those experiments gave new insights in molecular permeation and expression of multidrug resistance protein (P-gp) and multidrug resistance-associated protein (MRP).

Blood-air barrier

Lung epithelial cells (pneumocytes) cover alveoli in the lung. Their specific environment in form of a blood-air barrier can be simulated by use of a gradient perfusion container (Gueven et al. 1996). For this special purpose pneumocytes and endothelial cells were seeded for example on a polycarbonate filter and then transferred to a gradient perfusion culture container. During these experiments development of the tight junction complex was registered sealing in turn the blood-air barrier. Also typical features of polar differentiation within the epithelia were up-regulated. It was further shown that gradient perfusion culture in combination with pneumocytes and endothelial cells is a valuable model to investigate dose-controlled exposure of airborne particles. Finally, to elaborate characteristics of barrier transport and mechanisms of repair after alveolar injury a dose controlled air–liquid interface (ALI) was created by the use of A549 cells and kept in gradient perfusion culture (Tippe et al. 2002; Bitterle et al. 2006; Maier et al. 2008; Nandkumar et al. 2014).

Blood-gas barrier

A swim bladder assures that a fish can adjust its weight to the water pressure and in turn to float. Culture experiments with fish swim bladder gas gland were successfully performed by the application of a gradient perfusion container (Prem and Pelster 2000). In those experiments cells of gas gland were cultured on a filter at the interface between gas on one side and culture medium on the other side. The harvested epithelia showed a typical polarity and functionality as

it is known from the swim bladder gas gland in the living fish.

Testing new drugs

Orally administered drugs have to pass the epithelial barrier in the digestive tract before entering the interstitium within the organism. To test the transport of newly developed drugs across such an epithelial cell layer, long term gradient perfusion culture experiments were performed (Kloth et al. 1999, 2000). Experiments with Caco-2 cells in gradient perfusion culture demonstrated development of a tightly sealing epithelium cell layer. Further it was shown that in gradient perfusion culture reproducible results are achieved much earlier than observed in traditional 21 day static cultures. Also the permeability coefficient of several model medicines across a Caco-2 cell layer in gradient perfusion culture was approximately twofold higher than observed under static culture conditions (Masungi et al. 2004, 2009).

Renewal of epidermis/gingiva

The regeneration of epidermis and surgical repair of skin is an especially important subject in actual biomedicine. In order to evaluate a cost-effective engineering of full-thickness skin grafts and the treatment of ulcers, epidermis equivalents were investigated by the help of gradient perfusion culture (Kremer et al. 2001). In these experiments composite grafts of INTEGRA® matrix and human keratinocytes could be successfully generated in a gradient container. In a different context it was shown that development of a gingival epithelium (Lauer 2009; Hagedorn et al. 2009) or co-culture of keratinocytes and osteoblast-like cells in a perfusion container reveals much better results than obtained under static culture conditions (Glaum et al. 2010; Glaum and Wiedmann-Al-Ahmad 2013).

Regeneration of renal parenchyma

An increasing number of patients is suffering from acute and chronic kidney diseases. For this purpose the implantation of stem/progenitor cells and regeneration of damaged parenchyma are of special interest. Thus, to test developmental capacity renal, stem/progenitor cells were mounted between layers of a polyester fleece to simulate an artificial interstitium during perfusion culture (Minuth and Schumacher 2003; Minuth et al. 2004a, b, 2005b). Scanning electron microscopy (Fig. 4a), label by fluorescent Soybean Agglutinin (SBA) (Fig. 4b) and semi-thin sections (Fig. 4c) illustrate the successful generation of renal tubules during 13 days in perfusion culture (Heber et al. 2007; Hu et al. 2007; Minuth et al. 2007a).

Perfusion culture experiments in combination with an artificial interstitium and chemically defined media further showed that application of different kinds of polyester fleeces results in various patterns of spatial tubule development (Roessger et al. 2009). A new finding was that formation of tubules can be induced by aldosterone, while antagonists such as spironolactone or canrenoate prevent development (Minuth et al. 2007b, 2008, 2010a; Minuth and Denk 2008). When the contact between the mineralocorticoid receptor (MR) and heat shock protein 90 is disturbed by geldanamycin, formation of intact tubules is reduced, while atypical features arise in form of cell clusters.

At that time it was a fully new aspect in biomedicine that a polyester fleece used as an artificial interstitium can be principally applied for the regeneration of renal parenchyma (Blattmann et al. 2008; Minuth et al. 2009b). All up to date performed experiments yet point out that development of tubules is triggered by interactions between their basal lamina, newly synthesized fibers of the extracellular matrix and fibers of the polyester fleece (Minuth et al. 2010a, b, c, d; Miess et al. 2010; Glashauser et al. 2011).

However, performed experiments dealing with regeneration of renal parenchyma also inform that intact development of renal tubules is not self-evident but can be paralleled by arise of abnormal cell and extracellular matrix features, as it was recently detected (Minuth and Denk 2012, 2014a, b).

Finally, by keeping slices of adult kidney within a polyester interstitium during perfusion culture it became possible to investigate splicing of the Na–K–2Cl cotransporter NKCC2 adapted to typical renal environment (Schießl et al. 2013).

Stabilizing survival after transplantation

Before an implantation is made, stem/progenitor cells are normally kept in the beneficial atmosphere of a CO_2 dependent culture medium. In contrast, when an implantation has been performed, they are exposed to

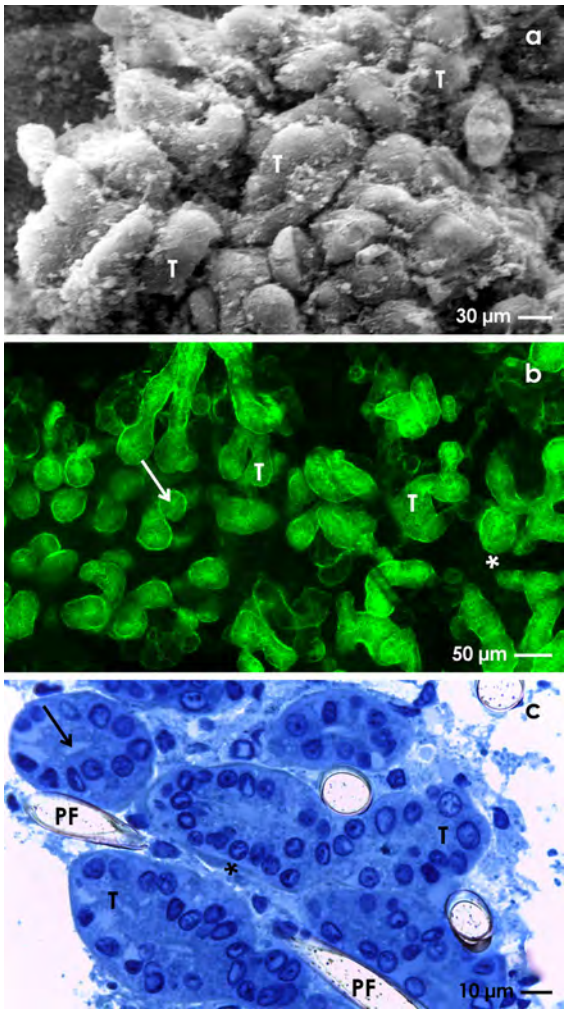


Fig. 4 Generation of renal tubules at the interface of a polyester interstitium after 13 days by perfusion culture. **a** Scanning electron microscopy demonstrates development of numerous renal tubules (T). **b** Fluorescent label for Soybean Agglutinin shows numerous tubules developing within an artificial interstitium. **c** Semithin section after Richardson staining shows generated tubules in oblique, respectively, vertical view between fibers of the polyester fleece (PF). Site of the basal lamina is marked by an *asterisk*, while lumen is indicated by an *arrow*

unbalanced interstitial fluid of diseased renal parenchyma. To investigate buffering of this harsh transition, renal stem/progenitor cells were exposed to conventional IMDM (Fig. 5a) in comparison to CO_2 Independent Medium (Fig. 5b) or Leibovitz's L-15 Medium (Fig. 5c) (Minuth et al. 2013; Minuth and Denk 2013). Analysis by transmission electron microscopy after fixation by conventional glutaraldehyde solution showed polar differentiation and typical features of transporting tubule cells. Formation of an excess of vacuoles as an indicator for toxicity was not observed. In so far the results demonstrate that CO_2 Independent Media or Leibovitz's L-15 Medium reflect an advantageous fluid microenvironment for isolation, implantation and initial development of renal stem/progenitor cells.

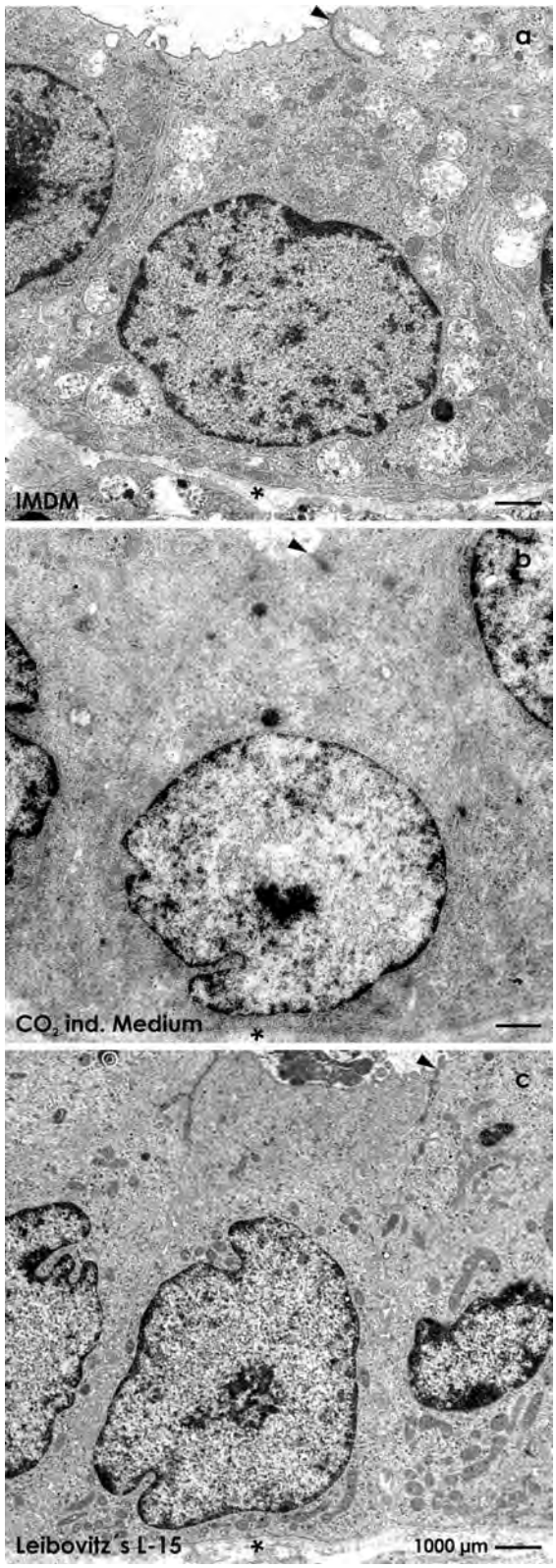
Engineering of connective tissue

A broad research field in regenerative medicine is the interaction between cells derived from connective tissue and a selected scaffold used as a substitute for extracellular matrix. In those culture set ups a variety of biodegradable biomaterials is applied. Especially in these experiments perfusion culture helps to prevent an overshoot of harmful metabolites by continuous elimination and keeps in turn fluid environment on a constant level.

Connective tissue barrier

Regarding connective tissue research it is barely considered that it can exhibit essential barrier functions. Experiments related to such barriers were performed for example with dentin discs mounted in a MINUSHEET® gradient perfusion container during culture (Schmalz et al. 1996, 1999, 2001, 2002; Camps et al. 2002; Galler et al. 2005; Demirci et al. 2008; Vajrabhaya et al. 2009; Ulker and Sengun 2009; Sengün et al. 2011; Ülker et al. 2013a, b; Kim et al. 2013a, b). In this series of experiments it was shown that polymerized dental resin materials release residual monomers, which may interact with pulp tissue. In so far gradient perfusion culture appears to be an appropriate technique for exploring long term toxic effects under realistic in vitro conditions (Sengün et al. 2011; Korsuwannawong et al. 2012; Kim et al. 2013a, b; da Silva et al. 2014). A further innovative approach is tooth regeneration that was investigated by allogeneic stem cells (Wei et al. 2013).

In addition, new information about permeability and degradation of gelatine membranes seeded with fibroblasts on one side was obtained by culture in a gradient perfusion container (Dreesmann et al. 2008). In a similar culture set up a cell-type specific four-component hydrogel was evaluated for the generation of hyaline cartilage and vertebral disc repair (Aberle et al. 2014).



◀**Fig. 5** Transmission electron microscopy demonstrates renal tubules generated at the interface of a polyester interstitium after 13 days of perfusion culture in **a** Iscove's Modified Dulbecco's Medium, **b** CO_2 Independent Medium and **c** Leibovitz's L-15 Medium. In all cases generated tubules exhibit a polarized epithelium. Neighboring cells are separated by a tight junction complex (*arrow head*). The basal lamina is indicated by an *asterisk*

Repair of hyaline cartilage

A challenge in tissue engineering is the treatment of cartilage defects by implantation of chondrocytes seeding within a biodegradable scaffold. The use of a MINUSHEET® tissue carrier has demonstrated that it can be a great help to investigate seeding of chondrocytes on selected scaffold materials. Moreover, it has been shown that use of a perfusion culture container improves cell biological quality of growing cartilage, when the medium is permanently renewed (Sittinger et al. 1994; Bujia et al. 1994, 1995; Sittinger et al. 1996, 1997). In those experiments basic data about the degradation process in various scaffold materials could be raised (Capitán Guarnizo et al. 2002). Knowing about exact kinetics of the degradation process a stepwise modification of scaffold materials became possible. As a result the risk of tissue repulsion after implantation was decreased by the application of those optimized scaffold materials (Rotter et al. 1998, 1999; Kreklau et al. 1999; Duda et al. 2000, 2004; Haisch et al. 2002; Gille et al. 2005). It was finally shown that electrospun polymer scaffolds have proven to be particularly advantageous (Schneider et al. 2011, 2012).

In this coherence it was also detected that application of natural extracellular matrix such as a collagen sponge does not improve the quality of generated cartilage (Fuss et al. 2000). In contrast, scaffold materials with modified polyethylene coating (Röpke et al. 2007) or a gelatine-based Spongostan® (Anders et al. 2009) revealed much more cartilage specific features than observed without surface treatment. In this context it was observed that synovial fibroblasts are able to adapt synthesis of extracellular matrix (Steinhagen et al. 2010). Finally, engineering of cartilage constructs by means of perfusion culture revealed to be an ideal model to investigate parameters affecting destructive joint diseases (Schultz et al. 1997; Risbud and Sittinger 2002; Bücheler and Haisch 2003).

Formation of bone

Not only for cartilage but also for bone engineering MINUSHEET® perfusion culture technique was successfully applied. For example, developmental capacity of osteoblasts and osteocytes was investigated with ceramic materials (Uemura et al. 2003; Wang et al. 2003; Leukers et al. 2005a; Yeatts and Fisher 2011; Bernhardt et al. 2011), decellularized spongeous bone (Seitz et al. 2007), collagen membranes (Rothamel et al. 2004) and mineralized collagen (Gelinsky et al. 2004; Bernhardt et al. 2008). Further hydroxyapatite scaffolds (Leukers et al. 2005b; Detsch et al. 2008; da Silva et al. 2010a, b), poly-d,l-lactic-co-glycolic acid (PLGA) sheets (Shearer et al. 2006), iron based metals (Quadbeck et al. 2010), bioactive glass (Yue et al. 2011), textile chitosan (Heinemann et al. 2008, 2009, 2010), 3D biphasic calcium phosphate scaffolds (Rath et al. 2012) and other biocorrodible bone replacement materials (Farack et al. 2011) were successfully applied in combination with perfusion culture.

Special focus was directed to production of optimized scaffold materials to stimulate cell colonization and formation of extracellular matrix (Mateescu et al. 2012; Campos et al. 2013). An important observation for clinical application was that bone development can be influenced by the process of sterilization, when scaffold material is applied consisting of poly(D,L-lactic-co-glycolic acid (PLGA) (Shearer et al. 2006). A further aspect was to elaborate by perfusion culture why formation of a biofilm occurs on titanium surfaces (Astasov-Frauenhoffer et al. 2012).

Osteoblasts in combination with a scaffold often form thick layers of tissue. A recurrent problem is that unstirred and consequently harming layers of fluid within growing tissue develop. For compensation the continuous provision with nutrition and oxygen must be substituted by transport of medium in pulses or by feedback loops so that bone constructs with an acceptable cell biological quality can develop (Volkmer et al. 2008, 2012).

Development of muscular tissue

Normally a car is not suitable for all terrains. In analogy, only three papers were found dealing with the regeneration of muscular tissue in combination with the MINUSHEET® perfusion culture system. In detail, when a layer of gastric mucosa was mounted in a tissue carrier and kept in a perfusion culture container, it was observed that not only gastric glands but also smooth muscular tissue are developing within the lamina propria (Kloth et al. 1998b). Further on, when formation of vessels in brain was investigated, the seeding of cerebral pericytes on selected biomaterials resulted in a high expression of site-specific pericytic aminopeptidase N/pAPN (Ramsauer et al. 1998). Finally, proliferation of smooth muscle cells was investigated on special electrospun polymer scaffolds (Rüder et al. 2012).

Generation of nervous tissue

A central problem in neurology research is the escape of dopamine synthesis during the course of Parkinson's disease. As a consequence, to investigate external influences on dopamine synthesis in mesencephalic neurons, MINUSHEET® perfusion culture was successfully performed (Blöchl and Sirrenberg 1996). For example, it was demonstrated that neurotrophins stimulate the release of dopamine via Trk and p75Lntr receptors. Further it could be demonstrated by perfusion culture with hippocampal neurons and cells of the pheochromacytoma cell line PC 12 that admixture of exogenous neurotrophins has positive feedback effects on secretion of synthesized neurotrophins. This pathway seems to be triggered by an activation of tyrosine kinase neurotrophin receptors (Canossa et al. 1997). It was further shown that alterations in sodium concentration play an important role in secretion of neurotrophins (Hoener 2000).

Perfusion culture was also applied to investigate differences in secretion between nerve growth factor and brain-derived neurotrophic factor (Griesbeck et al. 1999). Further SH-SY5Y human neuroblastoma cells exhibited differentiation into a neuronal-like state, when long term perfusion culture was applied (Constantinescu et al. 2007). In those experiments the cultures were kept for more than 2 months in an active state. In other series of experiments RAT-1 fibroblasts were investigated expressing Cypridina noctiluca luciferase (CLuc) driven by the promoter of the circadian clock gene Mma11 (Yamagishi et al. 2006). The experiments revealed that the CLuc reporter assay in combination with the applied perfusion culture is an

appropriate technique to test newly developed medications. Impressing results were obtained, when fish pituitary explants were kept in perfusion culture to investigate vasotocin and isotocin release (Kalamarz-Kubiak et al. 2011).

Maintenance of retina

Retina has a complex neural cell microarchitecture that is delimited by a pigment epithelium. Previous experiments have shown that typical morphological features cannot be maintained when culture is performed in static environment of a dish. For that reason intact retina was mounted in a tissue carrier to incubate it in a gradient perfusion culture container (Framme et al. 2002; Spiegel et al. 2002; Saikia et al. 2006; Jian and Jingbo 2007; Hamilton et al. 2007; Hammer et al. 2008; Kobuch et al. 2008). Those experiments revealed that retina neurons and the pigment epithelium maintain a perfect morphology for a culture period of at least 10 days. These new findings illustrate new perspectives for safety testing of newly developed pharmaceuticals designed for intraocular application.

In addition, these experiments give rise to new opportunities for investigating the wide field of retina inflammation, aging, degeneration and repair by the help of an adequate culture system (Klettner and Roider 2009; Klettner et al. 2009, 2012; Miura et al. 2010; Treumer et al. 2012. In this coherence for example molecular regulation of vascular endothelial growth factor secretion and cell biological reactions after fucoidan application were investigated (Klettner et al. 2013, 2014; Dithmer et al. 2014).

Conclusion

To improve the environment for cells and developing tissues under in vitro conditions, the MINUSHEET® perfusion culture system was developed 25 years ago. To stay versatile, a biomaterial for optimal cell adhesion is selected, mounted in a tissue carrier and then transferred to a 24 well culture plate. Seeding of cells is performed in static environment, while generation of tissue is made in various types of perfusion culture containers. To prevent an overshoot of paracrine factors a continuous transport of always fresh culture medium is performed. In the meantime numerous groups utilized the introduced system. A multitude of published papers illustrates that a variety of specialized tissues can be produced in an excellent cell biological quality urgently needed in tissue engineering, biomaterial research and advanced pharmaceutical drug testing.

Final remarks

In 1992 the project received the Philip Morris research award 'Challenge of the Future' in Munich/Germany.

To introduce developed tools on the market, Katharina Lorenz-Minuth founded non-profit-orientated Minucells and Minutissue Vertriebs GmbH (D-93077 Bad Abbach/Germany, www.minucells.com) by private sources.

Up to date more than 250 papers were published dealing with the MINUSHEET® perfusion culture system. A list of these different culture set ups is given in the data bank 'Proceedings in perfusion culture':

http://www.uni-regensburg.de/Anatomie/Minuth/proceedings.htm

For correct use of the MINUSHEET® perfusion culture system W.W. Minuth and L. Denk wrote a book entitled 'Advanced Culture Experiments with Adherent Cells: From single cells to specialized tissues in perfusion culture'. Open access publishing, University of Regensburg, 2011, ISBN Nr. 978-3-88246-355-2, 417 pages. URN: ubn:de:bvb:355-epub-313392. This manuscript can be downloaded as PDF file without costs and further obligations:

http://epub.uni-regensburg.de/31339/

Data raised by the MINUSHEET® perfusion culture system were earlier reviewed:

Minuth WW, Denk L, Glashauser A (2010) A modular culture system for the generation of multiple specialized tissues. Biomaterials 31:2945-2954

Minuth WW, Denk L (2012) Supportive development of functional tissues for biomedical research using the MINUSHEET® perfusion system. Clin Transl Med 1:22

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Conflict of interest A series of patents (DE 10 2004 054 125, DE 39 23 279, DE 42 00 446, DE 42 08 805, DE 44 43 902, DE

19 530 556, DE 196 48 876 C2, DE 199 52 847 B4, US 5 190 878, US 5 316 945, US 5 665 599, J 2847669, DE 10 2005 002 938, PA 10 2005 001 747.9) demonstrate that Will W. Minuth is the inventor of the MINUSHEET® perfusion culture system. W. W. Minuth and L. Denk declare no competing interests or financial conflicts.

References

Aberle T, Franke K, Rist E, Benz K, Schlosshauer B (2014) Cell-type specific four-component hydrogel. PLoS ONE 9:e86740

Aigner J, Kloth S, Kubitza M, Kashgarian M, Dermietzel R, Minuth WW (1994) Maturation of renal collecting duct cells in vivo and under perifusion culture. Epithel Cell Biol 3:70–78

Aigner J, Kloth S, Jennings ML, Minuth WW (1995) Transitional differentiation patterns of principal and intercalated cells during renal collecting duct development. Epithel Cell Biol 4:121–130

Anders JO, Mollenhauer J, Beberhold A, Kinne RW, Venbrocks RA (2009) Gelatin-based haemostyptic Spongostan as a possible three-dimensional scaffold for a chondrocyte matrix? An experimental study with bovine chondrocytes. J Bone Joint Surg Br 91:409–416

Astasov-Frauenhoffer M, Braissant O, Hauser-Gerspach I, Daniels AU, Weiger R, Waltimo T (2012) Isothermal microcalorimetry provides new insights into biofilm variability and dynamics. FEMS Microbiol Lett 337:31–37

Bakowsky U, Ehrhardt C, Loehbach C, Li P, Kneuer C, Jahn D, Hoekstra D, Lehr CM (2005) Adhesion molecule-modified cardiovascular prostheses: characterization of cellular adhesion in a cell culture model and by cellular force spectroscopy. In: Possart Wulff (ed) Adhesion: current research and applications. Wiley-VCH, Weinheim, pp 157–173

Bernhardt A, Lode A, Boxberger S, Pompe W, Gelinsky M (2008) Mineralised collagen—an artificial, extracellular bone matrix—improves osteogenic differentiation of bone marrow stromal cells. J Mater Sci Mater Med 19:269–275

Bernhardt A, Lode A, Peters F, Gelinsky M (2011) Optimization of culture conditions for osteogenically-induced mesenchymal stem cells in β-tricalcium phosphate ceramics with large interconnected channels. J Tissue Eng Regener Med 5:444–453

Bitterle E, Karg E, Schroeppel A, Kreyling WG, Tippe A, Ferron GA, Schmid O, Heyder J, Maier KL, Hofer T (2006) Dose-controlled exposure of A549 epithelial cells at the air–liquid interface to airborne ultrafine carbonaceous particles. Chemosphere 65:1784–1790

Blattmann A, Denk L, Strehl R, Castrop H, Minuth WW (2008) The formation of pores in the basal lamina of regenerated renal tubules. Biomaterials 29:2749–2756

Blöchl A, Sirrenberg C (1996) Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and p75Lntr receptors. J Biol Chem 271:21100–21107

Bruggeman LA, Doan RP, Loftis J, Darr A, Calabro A (2012) A cell culture system for the structure and hydrogel properties of basement membranes; application to capillary walls. Cell Mol Bioeng 5:194–204

Bücheler M, Haisch A (2003) Tissue engineering in otorhinolaryngology. DNA Cell Biol 22:549–564

Bujia J, Sittinger M, Hammer C, Burmester G (1994) Züchtung menschlichen Knorpelgewebes mit Hilfe einer Perfusionskammer (Culture of human cartilage tissue using a perfusion chamber). Laryngorhinootolgie 73:577–580

Bujia J, Sittinger M, Minuth WW, Hammer C, Burmester G, Kastenbauer E (1995) Engineering of cartilage tissue using bioresorbable polymer fleeces and perfusion culture. Acta Otolaryngol 115:307–310

Campos DM, Soares GA, Anselme K (2013) Role of culture conditions on in vitro transformation and cellular colonization of biomimetic HA-Col scaffolds. Biomatter 3:e24922

Camps J, About I, Thonneman B, Mitsiadis TA, Schmaltz G, Franquin JC (2002) Two- versus three-dimensional in vitro differentiation of human pulp cells into odontoblastic cells. Connect Tissue Res 43:396–400

Canossa M, Griesbeck O, Berninger B, Campana G, Kolbeck R, Thoenen H (1997) Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. Proc Natl Acad Sci USA 94:13279–13286

Capitán Guarnizo A, Viviente Rodriguez E, Osete Albaladejo JM, Torregrosa Carrasquer C, Diaz Manzano JA, Pérez-Mateos Cachá JA, Sprekelsen Gassó C (2002) Autoinjerto subcutáneo de cartilago neosintetizado utilizando el polimero ETHISORB® en conejos (Subcutaneous autograft with newly synthesized cartilage using ethisorb polymer in rabbits). Acta Otorrinolaringol Esp 53:631–636

Constantinescu R, Constantinescu AT, Reichmann H, Janetzky B (2007) Neuronal differentiation and long-term culture of the human neuroblastoma line SH-SY5Y. J Neural Transm Suppl 72:17–28

da Silva HM, Mateescu M, Damia C, Champion E, Soares G, Anselme K (2010a) Importance of dynamic culture for evaluating osteoblast activity on dense silicon-substituted hydroxyapatite. Colloids Surf B Biointerfaces 80:138–144

da Silva HM, Mateescu M, Ponche A, Damia C, Champion E, Soares G, Anselme K (2010b) Surface transformation of silicon-doped hydroxyapatite immersed in culture medium under dynamic and static conditions. Colloids Surf B Biointerfaces 75:349–355

da Silva JM, Rodrigues JR, Camargo CHR, Fernandes W Jr, Hiller KA, Schweikl H, Schmalz G (2014) Effectiveness and biological compatibility of different generations of dentin adhesives. Clin Oral Investig 18:607–613

Dankers PY, Boomker JM, Huizinga-van der Vlag A, Smedts FM, Harmsen MC, van Luyn MJ (2010) The use of fibrous, supramolecular membranes and human tubular cells for renal epithelial tissue engineering: towards a suitable membrane for a bioartificial kidney. Macromol Biosci 10:1345–1354

Dankers PY, Boomker JM, Huizinga-van der Vlag A, Wisse E, Appel WP, Smedts FM, Harmsen MC, Bosman AW, Meijer W, van Luyn MJ (2011) Bioengineering of living renal membranes consisting of hierarchical, bioactive supramolecular meshes and human tubular cells. Biomaterials 32:723–733

Demirci M, Hiller KA, Bosl C, Galler K, Schmalz G, Schweikl H (2008) The induction of oxidative stress, cytotoxicity, and genotoxicity by dental adhesives. Dent Mater 24:362–371

Detsch R, Uhl F, Deisinger U, Ziegler G (2008) 3D-Cultivation of bone marrow stromal cells on hydroxyapatite scaffolds fabricated by dispense-plotting and negative mould technique. J Mater Sci Mater Med 19:1491–1496

Dimova S, Vlaeminck V, Brewster ME, Noppe M, Jorissen M, Augustijns P (2005) Stable ciliary activity in human nasal epithelial cells grown in a perfusion system. Int J Pharm 292:157–168

Dithmer M, Fuchs S, Shi Y, Schmidt H, Richert E, Roider J, Klettner A (2014) Fucoidan reduces secretion and expression of vascular endothelial growth factor in the retinal pigment epithelium and reduces angiogenesis in vitro. PLoS ONE 9:e89150

Dreesmann L, Hajosch R, Ahlers M, Nuernberger JV, Schlosshauer B (2008) Permeability testing of biomaterial membranes. Biomed Mater 3:034119

Du Y, Han R, Wen F, Ng San San S, Xia L, Wohland T, Leo HL, Yu H (2008) Synthetic sandwich culture of 3D hepatocyte monolayer. Biomaterials 29:290–301

Duda GN, Haisch A, Endres M, Gebert C, Schroeder D, Hoffmann JE, Sittinger M (2000) Mechanical quality of tissue engineered cartilage: results after 6 and 12 weeks in vivo. J Biomed Mater Res 53:673–677

Duda GN, Kliche A, Kleemann R, Hoffmann JE, Sittinger M, Haisch A (2004) Does low-intensity pulsed ultrasound stimulate maturation of tissue-engineered cartilage? J Biomed Mater Res B Appl Biomater 68:21–28

Farack J, Wolf-Brandstetter C, Glorius S, Nies B, Standke G, Quadbeck P, Worch H, Scharnweber D (2011) The effect of perfusion culture on proliferation and differentiation of human mesenchymal cells on biocorrodible bone replacement material. Mater Sci Eng, B 176:1767–1772

Fiegel HC, Havers J, Kneser U, Smith MK, Moeller T, Kluth D, Mooney DJ, Rogiers X, Kaufmann PM (2004) Influence of flow conditions and matrix coatings on growth and differentiation of three-dimensionally cultured rat hepatocytes. Tissue Eng 10:165–174

Framme C, Kobuch K, Eckert E, Monzer J, Roider J (2002) RPE in perfusion tissue culture and its response to laser application. Ophthalmologica 216:320–328

Frerich B, Zückmantel K, Hemprich A (2006) Microvascular engineering in perfusion culture: immunohistochemistry and CLMS findings. Head Face Med 16:26

Frerich B, Zückmantel K, Winter K, Müller-Dürwald S, Hemprich A (2008) Maturation of capillary-like structures in a tube-like construct in perfusion and rotation culture. Int J Oral Maxillofac Surg 37:459–466

Fuss M, Ehlers EM, Russlies M, Rohwedel J, Behrens P (2000) Characteristics of human chondrocytes, osteoblasts and fibroblasts seeded onto a type I/III collagen sponge under different culture conditions. A light, scanning and transmission electron microscopy study. Ann Anat 182:303–310

Galler K, Hiller KA, Ettl T, Schmalz G (2005) Selective influence of dentin thickness upon cytotoxicity of dentin contacting materials. J Endod 31:396–399

Gelinsky M, König U, Sewing A, Pompe W (2004) Poröse Scaffolds aus mineralisiertem Kollagen—ein biomimetisches Knochenersatzmaterial. Mat-Wiss u Werkstofftech 35:229–233

Gille J, Meisner U, Ehlers EM, Müller A, Russlies M, Behrens P (2005) Migration pattern, morphology and viability of cells suspended in or sealed with fibrin glue: a histomorphologic study. Tissue Cell 37:339–348

Glashauser A, Denk L, Minuth WW (2011) Polyester fleeces used as an artificial interstitium influence the spatial growth of regenerating tubules. J Tissue Sci Eng 2:105

Glaum R, Wiedmann-Al-Ahmad M (2013) Cocultivation of human oral keratinocytes and human osteoblast-like cells. Methods Mol Biol 946:423–429

Glaum R, Wiedmann-Al-Ahmad M, Huebner U, Schmelzeisen R (2010) Tissue engineering of composite grafts: cocultivation of human oral keratinocytes and human osteoblast-like cells on laminin-coated polycarbonate membranes and equine collagen membranes under different culture conditions. J Biomed Mater Res A 93:704–715

Griesbeck O, Canossa M, Campana G, Gärtner A, Hoener MC, Nawa H, Kolbeck R, Thoenen H (1999) Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. Microsc Res Tech 45:262–275

Gueven N, Glatthaar B, Manke HG, Haemmerle H (1996) Cocultivation of rat pneumocytes and bovine endothelial cells on a liquid-air interface. Eur Respir J 9:968–975

Hagedorn GM, Blank A, Mai R, Weiland B, Spassov A, Lauer G (2009) Perfusion culture promotes differentiation of oral keratinocytes in vitro. J Physiol Pharmacol 60:25–29

Haisch A, Kläring S, Gröger A, Gebert C, Sittinger M (2002) A tissue-engineering model for the manufacture of auricular-shaped cartilage implants. Eur Arch Otorhinolaryngol 259:316–321

Hamilton RD, Leach L (2011) Isolation and properties of an in vitro human outer blood-retinal barrier model. Methods Mol Biol 686:401–416

Hamilton RD, Foss AJ, Leach L (2007) Establishment of a human in vitro model of the outer blood-retinal barrier. J Anat 211:707–716

Hammer M, Richter S, Kobuch K, Mata N, Schweitzer D (2008) Intrinsic tissue fluorescence in an organotypic perfusion culture of the porcine ocular fundus exposed to blue light and free radicals. Graefes Arch Clin Exp Ophtalmol 246:979–988

Hayashi M, Matsuzaki Y, Shimonaka M (2009) Impact of plasminogen on an in vitro wound healing model based on a perfusion cell culture system. Mol Cell Biochem 322:1–13

Heber S, Denk L, Hu K, Minuth WW (2007) Modulating the development of renal tubules growing in serum-free culture medium at an artificial interstitium. Tissue Eng 13:281–292

Heinemann C, Heinemann S, Bernhardt A, Worch H, Hanke T (2008) Novel textile chitosan scaffolds promote spreading, proliferation, and differentiation of osteoblasts. Biomacromolecules 9:2913–2920

Heinemann C, Heinemann S, Lode A, Bernhardt A, Worch H, Hanke T (2009) In vitro evaluation of textile chitosan

scaffolds for tissue engineering using human bone marrow stromal cells. Biomacromolecules 10:1305–1310

Heinemann C, Heinemann S, Bernhardt A, Lode A, Worch H, Hanke T (2010) In vitro osteoclastogenesis on textile chitosan scaffold. Eur Cell Mater 19:96–106

Herter P, Laube G, Gronczewski J, Minuth WW (1993) Silver-enhanced colloidal-gold labelling of rabbit kidney collecting-duct cell surfaces imaged by scanning electron microscopy. J Microsc 171:107–115

Hoener MC (2000) Role played by sodium in activity-dependent secretion of neurotrophins—revisited. Eur J Neurosci 12:3096–3106

Hu K, Denk L, de Vries U, Minuth WW (2007) Chemically defined medium environment for the development of renal stem cells into tubules. Biotechnol J 2:992–995

Jian GE, Jingbo LIU (2007) The stem cell and tissue engineering research in chinese ophalmology. Front Med China 1:6–10

Kalamarz-Kubiak H, Gozdowska M, Nietrzeba M, Kulcykowska E (2011) A novel approach to ATV and IT studies in fish brain and pituitary: in vitro perfusion technique. J Neurosci Methods 199:56–61

Kim MJ, Kim KN, Lee YK, Kim KM (2013a) Cytotoxicity test of dentin bonding agents using millipore filters as dentin substitutes in a dentin barrier test. Clin Oral Investig 17:1489–1496

Kim HS, Woo Chang S, Baek SH, Han SH, Lee Y, Zhu Q, Kum KY (2013b) Antimicrobial effect of alexidine and chlorhexidine against Enterococcus faecalis infection. Int J Oral Sci 5:26–31

Klettner A, Roider J (2009) Constitutive and oxidative-stress-induced expression of VEGF in the RPE are differently regulated by different Mitogen-activated protein kinases. Graefes Arch Clin Exp Ophthalmol 247:1487–1492

Klettner A, Kruse ML, Meyer T, Wesch D, Kabelitz D, Roider J (2009) Different properties of VEGF-antagonists: Bevacizumab but not Ranibizumab accumulates in RPE cells. Graefes Arch Clin Exp Ophthalmol 247:1601–1608

Klettner AK, Doths J, Roider J (2012) Nicotine reduces VEGF-secretion and phagocytotic activity in porcine RPE. Graefes Arch Clin Exp Ophthalmol 250:33–38

Klettner A, Westhues D, Lassen J, Bartsch S, Roider J (2013) Regulation of constitutive vascular endothelial growth factor secretion in retinal pigment epithelium/choroid organ cultures: p38, nuclear factor kappa B, and the vascular endothelial growth factor receptor-2/phosphatidylinositol 3 kinase pathway. Mol Vis 19:281–291

Klettner A, Recber M, Roider J (2014) Comparison of the efficacy of aflibercept, ranibizumab, and bevacizumab in an RPE/choroid organ culture. Graefes Arch Clin Exp Ophthalmol 252:1593–1598

Kloth S, Suter-Crazzolara C (2000) Modulation of renal blood vessel formation by glial cell line-derived neurotrophic factor. Microvasc Res 59:190–194

Kloth S, Schmidbauer A, Kubitza M, Weich HA, Minuth WW (1994) Developing renal microvasculature can be maintained under perfusion culture conditions. Eur J Cell Biol 63:84–95

Kloth S, Ebenbeck C, Kubitza M, Schmidbauer A, Röckl W, Minuth WW (1995) Stimulation of renal microvascular development under organotypic culture conditions. FASEB J 9:963–967

Kloth S, Gerdes J, Wanke C, Minuth WW (1998a) Basic fibroblast growth factor is a morphogenic modulator in kidney vessel development. Kidney Int 53:970–978

Kloth S, Eckert E, Klein SJ, Monzer J, Wanke C, Minuth WW (1998b) Gastric epithelium under organotypic perfusion culture. In Vitro Cell Dev Biol Anim 34:515–517

Kloth S, Kobuch K, Domokos J, Wanke C, Minuth WW (1999) Interactive tissue culture systems: Innovative tools for toxicity testing. BIOforum Int 3:70–72

Kloth S, Kobuch K, Domokos J, Wanke C, Monzer J (2000) Polar application of test substances in an organotypic environment and under continuous medium flow: a new tissue-based test concept for a broad range of applications in pharmacotoxicology. Toxicol In Vitro 14:265–274

Kobuch K, Herrmann WA, Framme C, Sachs HG, Gabel VP, Hillenkamp J (2008) Maintenance of adult porcine retina and retinal pigment epithelium in perfusion culture: characterisation of an organotypic in vitro model. Exp Eye Res 86:661–668

Korsuwannawong S, Srichan R, Vajrabhaya LO (2012) Cytotoxicity evaluation of self-etching dentine bonding agents in a cell culture perfusion condition. Eur J Dent 6:408–414

Kreklau B, Sittinger M, Mensing MB, Voigt C, Berger G, Burmester GR, Rahmanzadeh R, Gross U (1999) Tissue engineering of biphasic joint cartilage transplants. Biomaterials 20:1743–1749

Kremer M, Lang E, Berger A (2001) Organotypical engineering of differentiated composite-skin equivalents of human keratinocytes in a collagen-GAG matrix (INTEGRA Artificial Skin) in a perfusion culture system. Langenbecks Arch Surg 386:357–363

Lauer G (2009) Optimizing the mucosa graft: developing gingival keratinocyte-fibroblast construct. In: Meyer Ulrich, Meyer Thomas, Handschel Jörg, Wiesmann Hans Peter (eds) Fundamentals of tissue engineering and regenerative medicine, vol 27.6. Springer, Heidelberg, pp 375–380

Lehmann P, Kloth S, Aigner J, Dammer R, Minuth WW (1997) Lebende Langzeitkonservierung von humaner Gingiva in der Perfusionskultur (Vital long-term preservation of human gingiva in perfusion culture). Mund Kiefer Gesichtschir 1:26–30

Leukers B, Gülkan H, Irsen SH, Milz S, Tille C, Seitz H, Schieker M (2005a) Biocompatibility of ceramic scaffolds for bone replacement made by 3D printing. Mat-wiss u Werkstofftech 36:781–787

Leukers B, Gülkan H, Irsen SH, Milz S, Tille C, Schieker M, Seitz H (2005b) Hydroxyapatite scaffolds for bone tissue engineering made by 3D printing. J Mater Sci Mater Med 16:1121–1124

Maier KL, Alessandrini F, Beck-Speier I, Hofer TP, Diabaté S, Bitterle E, Stöger T, Jakob T, Behrendt H, Horsch M, Beckers J, Ziesenis A, Hültner L, Frankenberger M, Krauss-Etschmann S, Schulz H (2008) Health effects of ambient particulate matter-biological mechanisms and inflammatory responses to in vitro and in vivo particle exposures. Inhal Toxicol 20:319–337

Masungi C, Borremans C, Willems B, Mensch J, van Dijck A, Augustijns P, Brewster ME, Noppe M (2004) Usefulness of a novel Caco-2 cell perfusion system. I. In vitro prediction of the absorption potential of passively diffused compounds. J Pharm Sci 93:2507–2521

Masungi C, Mensch J, Willems B, van Dijck A, Borremans C, Noppe M, Brewster ME, Augustijns P (2009) Usefulness of a novel Caco-2 cell perfusion system II. Characterization of monolayer properties and peptidase activity. Pharmazie 64:36–42

Mateescu M, Rguitti E, Ponche A, Descamps M, Anselme K (2012) Biomimetic evaluation of β tricalcium phosphate prepared by hot isostatic pressing. Biomatter 2:103–113

Miess C, Glashauser A, Denk L, deVries U, Minuth WW (2010) The interface between generating renal tubules and a polyester fleece in comparison to the interstitium of the developing kidney. Ann Biomed Eng 38:2197–2209

Minuth WW (1990) Methode zur Kultivierung von Zellen. Patent DE-PS 3923279

Minuth WW, Denk L (2008) Generierung von Tubuli aus renalen Stamm-/Progenitorzellen mit Aldosteron (Aldosterone-dependent generation of tubules derived from renal stem/progenitor cells). Transplantationsmedizin 20:47–52

Minuth WW, Denk L (2012) Interstitial interfaces show marked differences in regenerating tubules, matured tubules, and the renal stem/progenitor cell niche. J Biomed Mater Res A 100:1115–1125

Minuth WW, Denk L (2013) Initial steps to stabilize the microenvironment for implantation of stem/progenitor cells in diseased renal parenchyma. Transpl Technol 1:2

Minuth WW, Denk L (2014a) Tannic acid label indicates abnormal cell development coinciding with regeneration of renal tubules. BMC Clin Pathol 14:34

Minuth WW, Denk L (2014b) Detection of abnormal extracellular matrix in the interstitium of regenerating renal tubules. Int J Mol Sci 15:23240–23254

Minuth WW, Rudolph U (1990) A compatible support system for cell culture in biomedical research. Cytotechnology 4:181–189

Minuth WW, Schumacher K (2003) Von der renalen Stammzellnische zum funktionellen Tubulus (From the renal stem cell niche to functional tubule). Med Klin (Munich) 98:31–35

Minuth WW, Stöckl G, Kloth S, Dermietzel R (1992a) Construction of an apparatus for perfusion cell cultures which enables in vitro experiments under organotypic conditions. Eur J Cell Biol 57:132–137

Minuth WW, Dermietzel R, Kloth S, Hennerkes B (1992b) A new method culturing renal cells under permanent superfusion and producing a luminal-basal medium gradient. Kidney Int 41:215–219

Minuth WW, Fietzek W, Kloth S, Aigner J, Herter P, Röckl W, Kubitza M, Stöckl G, Dermietzel R (1993) Aldosterone modulates PNA binding cell isoforms within renal collecting duct epithelium. Kidney Int 44:537–544

Minuth WW, Kloth S, Majer V, Dermietzel R (1994) Growth of MDCK cells on non-transparent supports. In Vitro Cell Dev Biol Anim 30A:12–14

Minuth WW, Aigner J, Kloth S, Steiner P, Tauc M, Jennings ML (1997a) Culture of embryonic renal collecting duct epithelia in a gradient container. Pediatr Nephrol 11:140–147

Minuth WW, Steiner P, Strehl R, Kloth S, Tauc M (1997b) Electrolyte environment modulates differentiation in embryonic renal collecting duct epithelia. Exp Nephrol 5:414–422

Minuth WW, Steiner P, Strehl R, Schumacher K, de Vries U, Kloth S (1999) Modulation of cell differentiation in perfusion culture. Exp Nephrol 7:394–406

Minuth WW, Strehl R, Schumacher K, de Vries U (2001) Long term culture of epithelia in a continuous fluid gradient for biomaterial testing and tissue engineering. J Biomater Sci Polym Ed 12:353–365

Minuth WW, Strehl R, Schumacher K (2004a) Tissue factory: conceptual design of a modular system for the in vitro generation of functional tissues. Tissue Eng 10:285–294

Minuth WW, Sorokin L, Schumacher K (2004b) Generation of renal tubules at the interface of an artificial interstitium. Cell Physiol Biochem 14:387–394

Minuth WW, Schumacher K, Strehl R (2005a) Renal epithelia in long term gradient culture for biomaterial testing and tissue engineering. Biomed Mater Eng 15:51–63

Minuth WW, Denk L, Heber S (2005b) Growth of embryonic renal parenchyme at the interphase of a polyester artificial interstitium. Biomaterials 26:6588–6598

Minuth WW, Denk L, Hu K (2007a) The role of polyester interstitium and aldosterone during structural development of renal tubules in serum-free medium. Biomaterials 28:4418–4428

Minuth WW, Denk L, Hu K, Castrop H, Gomez-Sanchez C (2007b) The tubulogenic effect of aldosterone is attributed to intact binding and intracellular response of the mineralocorticoid receptor. CEJB 2:307–325

Minuth WW, Blattmann A, Denk L, Castrop H (2008) Mineralocorticoid receptor, heat shock proteins and immunophilins participate in the transmission of the tubulogenic signal of aldosterone. J Epithel Biol Pharmacol 1:24–34

Minuth WW, Denk L, Roessger A (2009a) Gradient perfusion culture—simulating a tissue-specific environment for epithelia in biomedicine. J Epithelial Biol Pharmacol 2:1–13

Minuth WW, Denk L, Meese C, Rachel R, Roessger A (2009b) Ultrastructural insights in the interface between generated renal tubules and a polyester interstitium. Langmuir 25:4621–4627

Minuth WW, Denk L, Glashauser A (2010a) Promoting and harmful effects of steroid hormones on renal stem/progenitor cell development. J Tissue Sci Eng 1:101

Minuth WW, Denk L, Roessger A (2010b) Regenerating tubules for kidney repair. In: Artmann Gerhard M, Minger Stephen, Hescheler Jürgen (eds) Stem cell engineering: principals and applications. Springer, Heidelberg, pp 321–344

Minuth WW, Denk L, Glashauser A (2010c) Towards a guided regeneration of renal tubules at a polyester interstitium. Materials 3:2369–2392

Minuth WW, Denk L, Glashauser A (2010d) Cell and drug delivery therapeutics for controlled renal parenchyma regeneration. Adv Drug Deliv Rev 62:841–854

Minuth WW, Denk L, Gruber M (2013) Search for chemically defined culture medium to assist initial regeneration of diseased renal parenchyma after stem/progenitor cell implantation. Int J Stem Cell Res 1:202

Miura Y, Klettner A, Noelle B, Hasselbach H, Roider J (2010) Change of morphological and functional characteristics of retinal pigment epithelium cells during cultivation of retinal pigment epithelium-choroid perfusion tissue culture. Ophthalmic Res 43:122–133

Miura Y, Huettmann G, Orzekowsky-Schroeder R, Steven P, Szaszák M, Koop N, Brinkmann R (2013) Two-photon microscopy and fluorescence lifetime imaging of retinal pigment epithelial cells under oxidative stress. Invest Ophthalmol Vis Sci 54:3366–3377

Mollet BB, Comellas-Aragonès M, Spiering AJH, Söntjens SHM, Meijer EW, Dankers PYW (2014) A modular approach to easily processable supromolecular bilayered scaffolds with tailorable properties. J Mater Chem B 2:2483–2493

Nandkumar MA, Ashna U, Thomas LV, Nair PD (2014) Pulmonary surfactant expression analysis—role of cell-cell interactions and 3-D tissue-like architecture. Cell Biol Int. doi:10.1002/cbin.10389

Prem C, Pelster B (2000) Swimbladder gas gland cells of the European eel cultured in superfusion system. Methods Cell Sci 22:125–132

Quadbeck P, Hauser R, Kümmel K, Standke G, Stephani G, Nies B, Rößler S, Wegener B (2010) Iron based cellular metals for degradable synthetic bone replacement. PM2010 World Congress, PM Biomaterials, vol 4. Florenz, Italy, p 747

Ramsauer M, Kunz J, Krause D, Dermietzel R (1998) Regulation of a blood-brain barrier-specific enzyme expressed by cerebral pericytes (pericytic aminopeptidase N/pAPN) under cell culture conditions. J Cereb Blood Flow Metab 18:1270–1281

Rath SN, Strobel LA, Arkudas A, Beier JP, Maier AK, Greil P, Horch RE, Kneser U (2012) Osteoinduction and survival of osteoblasts and bone-marrow stromal cells in 3D biphasic calcium phosphate scaffolds under static and dynamic culture conditions. J Cell Mol Med 16:2350–2361

Reischl J, Prelle K, Schöl H, Neumüller C, Einspanier R, Sinowatz F, Wolf E (1999) Factors affecting proliferation and dedifferentiation of primary bovine oviduct epithelial cells in vitro. Cell Tissue Res 296:371–383

Risbud MV, Sittinger M (2002) Tissue engineering: advances in in vitro cartilage generation. Trends Biotechnol 20:351–356

Roessger A, Denk L, Minuth WW (2009) Potential of stem/progenitor cell cultures within polyester fleeces to regenerate renal tubules. Biomaterials 30:3723–3732

Röpke E, Schön I, Vogel J, Jamali J, Bloching M, Berghaus A (2007) Screening von modifizierten Polyethylenoberflächen für das Tissue-Engineering von Chondrozyten (Screening of modified polyethylene surfaces for tissue engineering of chondrocytes). Laryngorhinootologie 86:37–43

Rothamel D, Schwarz F, Sculean A, Herten M, Scherbaum W, Becker J (2004) Biocompatibility of various collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells. Clin Oral Implants Res 15:443–449

Rotter N, Aigner J, Naumann A, Planck H, Hammer C, Burmester G, Sittinger M (1998) Cartilage reconstruction in head and neck surgery: comparison of resorbable polymer scaffolds for tissue engineering of human septal cartilage. J Biomed Mater Res 42:347–356

Rotter N, Aigner J, Naumann A, Hammer C, Sittinger M (1999) Behavior of tissue-engineered human cartilage after transplantation into nude mice. J Mater Sci Mater Med 10:689–693

Rüder C, Sauter T, Becker T, Kratz K, Hiebl B, Jung F, Lendlein A, Zohlnhöfer D (2012) Viability, proliferation and adhesion of smooth muscle cells and human umbilical vein endothelial cells on electrospun polymer scaffolds. Clin Hemorheol Microcirc 50:101–112

Saikia P, Maisch T, Kobuch K, Jackson TL, Bäumler W, Szeimies RM, Gabel VP, Hillenkamp J (2006) Safety testing of indocyanine green in an ex vivo porcine retina model. Invest Ophthalmol Vis Sci 47:4998–5003

Schießl IM, Rosenauer A, Kattler V, Minuth WW, Oppermann M, Castrop H (2013) Dietary salt intake modulates differential splicing of the Na–K–2Cl cotransporter NKCC2. Am J Physiol Renal Physiol 305:F1139–F1148

Schmalz G, Garhammer P, Schweiki H (1996) A commercially available cell culture device modified for dentin barrier tests. J Endod 22:249–252

Schmalz G, Schuster U, Nuetzel K, Schweikl H (1999) An in vitro pulp chamber with three-dimensional cell cultures. J Endod 25:24–29

Schmalz G, Schuster U, Thonemann B, Barth M, Esterbauer S (2001) Dentin barrier test with transfected bovine pulp-derived cells. J Endod 27:96–102

Schmalz G, Schuster U, Koch A, Schweikl H (2002) Cytotoxicity of low pH dentin-bonding agents in a dentin barrier test in vitro. J Endod 28:188–192

Schneider T, Kohl B, Sauter T, Becker T, Kratz K, Schossig M, Hiebl B, Jung F, Lendlein A, Ertel W, Schulze-Tanzil G (2011) Viability, adhesion and differentiated phenotype of articular chondrocytes on degradable polymers and electro-spun structures thereof. Macromol Symp 309–310:28–39

Schneider T, Kohl B, Sauter T, Kratz K, Landlein A, Ertel W, Schulze-Tanzil G (2012) Influence of fiber orientation in electrospun polymer scaffolds on viability, adhesion and differentiation of articular chondrocytes. Clin Hemorheol Microcirc 52:325–336

Schultz O, Keyszer G, Zacher J, Sittinger M, Burmester GR (1997) Development of in vitro model systems for destructive joint diseases: novel strategies for establishing inflammatory pannus. Arthritis Rheum 40:1420–1428

Schumacher K, Strehl R, Kloth S, Tauc M, Minuth WW (1999) The influence of culture media on embryonic renal collecting duct cell differentiation. In Vitro Cell Dev Biol Anim 35:465–471

Schumacher K, Strehl R, de Vries U, Minuth WW (2002a) Advanced technique for long term culture of epithelia in a continuous luminal-basal medium gradient. Biomaterials 23:805–815

Schumacher K, Castrop H, Strehl R, de Vries U, Minuth WW (2002b) Cyclooxygenases in the collecting duct of neonatal rabbit kidney. Cell Physiol Biochem 12:63–74

Schumacher K, Khong YM, Chang S, Ni J, Sun W, Yu H (2007) Perfusion culture improves the maintenance of cultured liver tissue slices. Tissue Eng 13:197–205

Seitz S, Ern K, Lamper G, Docheva D, Drosse I, Milz S, Mutschler W, Schieker M (2007) Influence of in vitro cultivation on the integration of cell-matrix constructs after subcutaneous implantation. Tissue Eng 13:1059–1067

Sengün A, Yalcin M, Ülker HE, Öztürk B, Hakki SS (2011) Cytotoxicity evaluation of dentin bonding agents by dentin

barrier test on 3-dimensional pulp cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 112:e83–e88

Shearer H, Ellis MJ, Perera SP, Chaudhuri JB (2006) Effects of common sterilization methods on the structure and properties of poly(D, L lactic-co-glycolic acid) scaffolds. Tissue Eng 12:2717–2727

Sittinger M, Bujia J, Minuth WW, Hammer C, Burmester GR (1994) Engineering of cartilage tissue using bioresorbable polymer carriers in perfusion culture. Biomaterials 15:451–456

Sittinger M, Bujia J, Rotter N, Reitzel D, Minuth WW, Burmester GR (1996) Tissue engineering and autologous transplant formation: practical approaches with resorbable biomaterials and new cell culture techniques. Biomaterials 17:237–242

Sittinger M, Schultz O, Keyszer G, Minuth WW, Burmester GR (1997) Artificial tissues in perfusion culture. Int J Artif Organs 20:57–62

Spiegel D, Schefthaler M, Kobuch K (2002) Outflow facilities through Descemet's membrane in rabbits. Graefes Arch Clin Exp Ophatalmol 240:111–113

Steiner P, Strehl R, Kloth S, Tauc M, Minuth WW (1997) In vitro development and preservation of specific features of collecting duct epithelial cells from embryonic rabbit kidney are regulated by the electrolyte environment. Differentiation 62:193–202

Steinhagen J, Bruns J, Niggemeyer O, Fuerst M, Rüther W, Schünke M, Kurz B (2010) Perfusion culture system: synovial fibroblasts modulate articular chondrocyte matrix synthesis in vitro. Tissue Cell 42:151–157

Sternberg K, Selent C, Hakansson N, Töllner J, Langer T, Seiter H, Schmitz KP (2004) Bioartifizielle Materialien in der Urologie (Bioartificial materials in urology). Urologe A 43:1200–1207

Steuer H, Jaworski A, Stoll D, Schlosshauer B (2004) In vitro model of the outer blood-retina barrier. Brain Res Brain Res Protoc 131:26–36

Steuer H, Jaworski A, Elger B, Kaussmann M, Keldenich J, Schneider H, Stoll D, Schlosshauer B (2005) Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. Invest Ophthalmol Vis Sci 46:1047–1053

Strehl R, Schumacher K, Minuth WW (2004) Controlled respiratory gas delivery to embryonic renal epithelial explants in perfusion culture. Tissue Eng 10:1196–1203

Tiemann U, Bücher K, Pfarrer Ch, Pöhland R, Becker F, Kanitz W, Schmidt P (2005) Influence of ovarian steroid hormones or platelet-activating factor on mRNA of platelet-activating factor receptor in endometrial explant perfusion cultures from ovariectomized bovine. Prostaglandins Other Lipid Mediat 76:35–47

Tippe A, Heinzmann U, Roth C (2002) Deposition of fine and ultrafine aerosol particles during exposure at the air/cell interface. J Aerosol Sci 33:207–218

Treumer F, Klettner A, Baltz J, Hussain AA, Brinkmann R, Roider J, Hillenkamp J (2012) Vectorial release of matrix metalloproteinases (MMPs) from porcine RPE-choroid explants following selective retina therapy (SRT): towards slowing the macular ageing process. Exp Eye Res 97:63–72

Uemura T, Dong J, Wang Y, Kojima H, Saito T, Iejima D, Kikuchi M, Tanaka J, Tateishi T (2003) Transplantation of cultured bone cells using combinations of scaffolds and culture techniques. Biomaterials 24:2277–2286

Ulker HE, Sengun A (2009) Cytotoxicity evaluation of self adhesive composite resin cements by dentin barrier test on 3D pulp cells. Eur J Dent 3:120–126

Ülker HE, Ülker M, Gümüs HÖ, Yalcin M, Sengün A (2013a) Cytotoxicity testing of temporary luting cements with two- and three-dimensional cultures of bovine dental pulp-derived cells. Biomed Res Int 2013:910459

Ülker H, Ulker M, Botsali M, Dündar A, Acar H (2013b) Cytotoxicity evaluation of dentin contacting materials with dentin barrier test device using erbium-doped yttrium, aluminium, and garnet laser-treated dentin. Hum Exp Toxicol 33:949–955

Vajrabhaya L, Korsuwannawong S, Bosl C, Schmalz G (2009) The cytotoxicity of self-etching primer bonding agents in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107:e86–e90

Volkmer E, Drosse I, Otto S, Stangelmayer A, Stengele M, Kallukalam BC, Mutschler W, Schieker M (2008) Hypoxia in static and dynamic 3D culture systems for tissue engineering of bone. Tissue Eng Part A 14:1331–1340

Volkmer E, Otto S, Polzer H, Saller M, Trappendreher D, Zagar D, Hamisch S, Ziegler G, Wilhelmi A, Mutschler W, Schieker M (2012) Overcoming hypoxia in 3D culture systems for tissue engineering of bone in vitro using an automated, oxygen-triggered feedback loop. J Mater Sci Mater Med 23:2793–2801

Wang Y, Uemura T, Dong J, Kojima H, Tanaka J, Tateishi T (2003) Application of perfusion culture system improves in vitro and in vivo osteogenesis of bone marrow-derived osteoblastic cells in porous ceramic materials. Tissue Eng 9:1205–1214

Wei F, Song T, Ding G, Xu J, Liu Y, Liu D, Fan Z, Zhang C, Shi S, Wang S (2013) Functional tooth restoration by allogeneic mesenchymal stem cell-based bio-root regeneration in swine. Stem Cells Dev 22:1752–1762

Wu Z, Zhou Q, Duan H, Wang X, Xiao J, Duan H, Li N, Li C, Wan P, Liu Y, Song Y, Zhou C, Huang Z, Wang Z (2014) Reconstruction of auto-tissue-engineered lamellar cornea by dynamic culture for transplantation: a rabbit model. PLoS ONE 9:e93012

Xia L, Ng S, Han R, Tuo X, Xiao G, Leo HL, Cheng T, Yu H (2009) Laminar-flow immediate-overlay hepatocyte sandwich perfusion system for drug hepatotoxicity testing. Biomaterials 30:5927–5936

Yamagishi K, Enomoto T, Ohmiya Y (2006) Perfusion-culture-based secreted bioluminescence reporter assay in living cells. Anal Biochem 345:15–21

Yeatts AB, Fisher JP (2011) Bone tissue engineering bioreactors: dynamic culture and the influence of shear stress. Bone 48:171–181

Yue S, Lee PD, Poologasundarampillai G, Jones JR (2011) Evaluation of 3-D bioactive glass scaffolds dissolution in a perfusion flow system with X-ray microtomography. Acta Biomater 7:2637–2643

Zheng W, Jimenez-Linan M, Rubin BS, Halvorson LM (2007) Anterior pituitary gene expression with reproductive aging in the female rat. Biol Reprod 76:1091–1102