Cell Tissue Res (1998) 291:1–11

REVIEW

Will W. Minuth · Michael Sittinger · Sabine Kloth

Tissue engineering: generation of differentiated artificial tissues for biomedical applications

Received: 17 April 1997 / Accepted: 27 May 1997

Abstract A new field in biomedical science has been established. Cell biologists, engineers, and surgeons now work within a team. Artificial connective, epithelial, or neuronal tissues are being constructed using living cells and different kinds of biomaterials. Numerous companies and laboratories are presenting dynamic developments in this field. Prognoses predict that, at the beginning of the coming century, the industry of tissue engineering will reach the importance of the present genetic technology. An enormous demand for organ and tissue transplants motivates research activities and drives the acquisition of innovative techniques and creative solutions. At the front of this development is the creation of artificial skin for severely burned patients and the generation of artificial cartilage for implantation in articular joint diseases. Future challenges are the construction of liver organoids and the development of an artificial kidney on the basis of cultured cells. In this paper we show strategies, needs, tools, and equipment for tissue engineering. The presupposition for all projects is the induction, development, and maintenance of differentiation within the tissue under in vitro conditions. As experiments in conventional culture dishes continued to fail, new cell and tissue culture methods had to be developed. Tissues are cultured under conditions as close as possible to their natural environment. To optimize adherence or embedding, cells are grown on novel tissue carriers and on individually selected biomatrices or scaffolds. The tissues are subsequently transferred into different types of containers for permanent perfusion with fresh culture medium. This guarantees constant nutrition of the developing tissue and prevents the accumulation of harmful metabolites. An organo-typical environment for epithelial cells, for example, is obtained in gradient containers, which are permanently superfused at the apical and basal sides with different media. Long term experiments result in cultured tissues in a quality thus far unreached.

Key words Tissue engineering · Differentiation · Organotypical environment · Perfusion culture

The experimental work was supported by the 'Deutsche Forschungsgemeinschaft' (Mi 331/4–2, Bu445/5–1, Kl 741/7–1).

W. W. Minuth (✉) · S. Kloth
University of Regensburg, Institute of Anatomy,
University Street 31, D-93053 Regensburg, Germany
Fax: 49 (0)941 943 2868;
E-mail: will.minuth@vkl.uni-regensburg.de

M. Sittinger
German Rheumatism Research Center and Department
of Medicine III, Charité, Humboldt University of Berlin, Germany

Introduction

A new field in biomedical science has been established. Cell biologists, engineers, and surgeons now work within a team. Artificial connective, epithelial, or neuronal tissues are being constructed using living cells and a broad variety of biomaterials (Service 1995).

The aim

The enormous need for 'spare parts' for the human body is the driving force for research and requires innovative techniques and creative solutions. The development of artificial skin for severely burned patients is among the most advanced tissue-engineering attempts (Boyce et al. 1995; von Donnersmark et al. 1995; Rennekampff et al. 1996). Intensive research is being focused on the generation of artificial cartilage and bone (Bizios 1994; Frangos and Hillsley 1993; Oliva et al. 1996) to treat, e.g., articular joint diseases or injuries (Reddi 1994; Glowacki and Mizuno 1996) or augment defects of the neck, nose, and ear in plastic surgery (Puelacher et al. 1994; Sittinger et al. 1996a, b). Future challenges are the construction of liver organoids for bridging comas (Gerlach et al. 1994a, b; Jauregui et al. 1996a, b; Naik et al. 1996) or the development of an artificial kidney based on cultured cells (Humes 1996a, b).

Vision and reality

Tissue engineering combines living cells with a wide range of selected biomaterials, mostly as a substitute for the extracellular matrix or the stroma of tissues or organs. In almost any approach, the biocompatibility of the artificial extracellular matrix is a major point of discussion. This aspect is especially important for the development of autologous transplants. Only typical molecular structures of tissues should be expressed to avoid any immunological response of the immune system. Numerous experiments have shown that cells need an appropriate biomatrix or scaffold as an optimal cell support to develop their typical differentiated phenotype in vitro. Such human tissues formed in vitro can be applied as interacting tissues to develop advanced disease models (Schmalz et al. 1996; Schulz et al. 1996). This technical approach may also have great potential for replacing many harmful experiments on animals. Novel carriers for tissues and innovative cell-culture techniques provide the basic conditions needed to develop the complex structure of tissues and organoids. For the first time in the field of biomaterial research, chronic inflammatory disease and chronic intoxication in vitro long-term experiments have become possible.

Economic potential of engineered autologous transplants

The yearly cost caused by defects and failure of organs or tissues are $ 400 billion in the USA (Nerem and Sambanis 1995). Today, about 10 million Americans are living with implants. About 140 000 artificial hip joints are implanted per year. The yearly cost for implants in all surgical fields is estimated at $ 30 billion. In Germany only inofficial numbers are given. There are 20 000 knee prostheses implanted per year and more than 100 000 patients with relevant defects in joint cartilage are known. Up to now, destructed hip joints are replaced by metal prostheses, for blood-vessels and the urinary system implants made of different polymeric materials are applied (Gebelein 1993; Bizios 1994; Desgrandchamps et al. 1995; Schmalzried et al. 1996; Wilczek et al. 1996). These implants usually have major problems, such as loosening, degradation, inflammation, as well as other complications such as blood clotting. In the future, one would prefer to create tissues extracorporally from cultivated cells and subsequently implant the construct. Tissue engineering offers this fascinating new possibility. It is based on the idea of seeding the patient's own cells into suitable biomatrices or scaffolds to control their proliferation and their three-dimensional spreading. The tissue-typical differentiation is specifically influenced. The novel techniques allow the creation of defined three-dimensional tissues and organoid structures.

Tissue engineering requires new methodological approaches

The creation of functional tissues depends on innovative culture setups. It is important to consider that functional tissue structures are only able to develop if the histotypical sociality of the respective cell types is taken into account. Usually the interstitial three-dimensional structure is provided by a scaffolding of biomaterial, which is either stable or resorbed after weeks or months and replaced by the specific synthesis of extracellular tissue. Resorbable compounds made of polylactic acid and polyglycolic acid are frequently used (Sittinger et al. 1996b). Here the settlement of the specific cell type is the first important step. Previous experiments showed that only on an optimal biomatrix makes it possible to develop an optimum of functional differentiation. The later degradation of the resorbable polymers may release α-hydroxy acids in rather high local concentrations. This leads to a local acidification of the environment and possibly to a damaging effect on the generated tissue. For that reason, conventional cell and tissue dishes should not be used since they lack a continuous exchange of medium. Perfusion culture systems guarantee a constant removal of metabolite waste (Fig. 1). Finally, culture media supporting cell differentiation rather than proliferation have to be used.

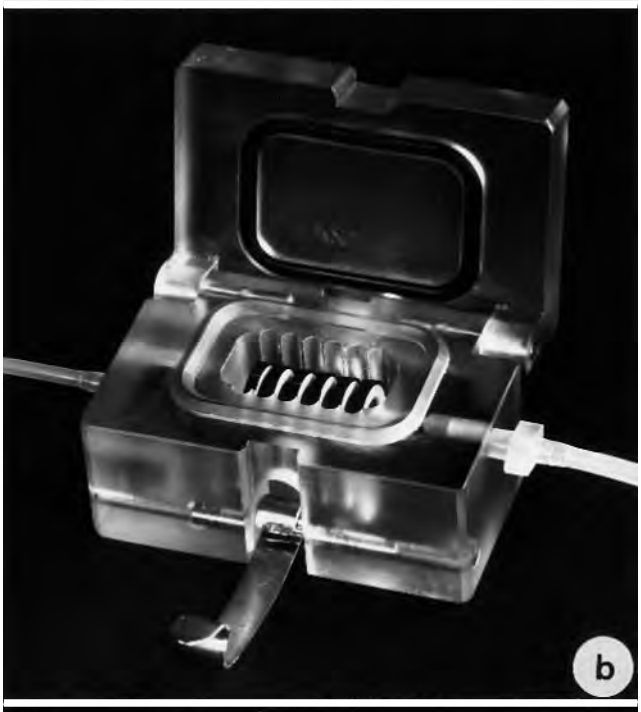
Tools for tissue engineering

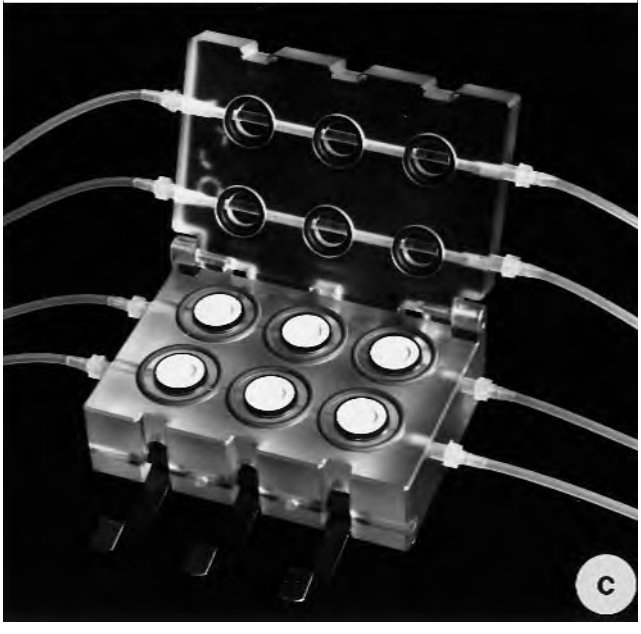
As present experience shows, tissue engineering must be done in three principal steps (Table 1). The first step of tissue engineering, the reproduction of cells, is done in conventional culture dishes. However, for starting and maintaining the differentiation, novel tissue carriers and perfusion culture containers have to be used (Table 2) (Minuth et al. 1992; Bujia et al. 1995). The first step comprises sufficient multiplication, proliferation, and spreading of the cells. The second step is the seeding of the reproduced cells on a suitable tissue support, biomatrix or scaffold. On the one hand, this biomatrix is an important requirement for cells to spead three-dimensionally; on the other hand, it is the basis of an optimal differentiation of tissues (Minuth

Table 1 Tissue engineering – three experimental steps are necessary for differentiation

	1st step	2nd step	3rd step
Necessities	Multiplication, proliferation, spreading	Induction of differentiation	Maintaining the phenotype
Methods	Culture dish[a]	Tissue carrier optimizing the matrix[b]	Stabilized environment with perfusion culture[c, d]

[a] Koechlin et al. (1991)
[b] Minuth et al. (1994)
[c] Minuth et al. (1992)
[d] Kloth et al. (1995)





et al. 1995, 1996; Sittinger et al. 1996a; Harvey et al. 1996). The third step aims towards the longterm maintenance of the differentiated phenotype of the engineered tissues.

In order to handle the cultures, an optimal biomatrix is selected and placed in tissue carriers (Fig. 1a). For initiation and maintenance of differentiation, the tissue carriers are transferred into culture containers that are permanently perfused with fresh medium (Fig. 2b,c). The optimum of environmental conditions reached in those containers again influences the differentiation of the tissue. The cul-

Table 2 Differentiation of cells and tissues is triggered at different levels. Tissue-specific differentiation is obtained by a synergistic action

Degree of differentiation	Necessities	Realisations	Literature
	Optimal anchorage for differentiation	Filters, fleeces, biomatrices	[a, b, c]
	Optimizing the extra-cellular matrix	Coating with extracellular proteins	[d, e, f]
	Cell and tissue transportation	Tissue carrier for optimal handling	[a]
	Elimination of metabolites/maintaining paracrine factors on a constant level	Perfusion culture containers	[g]
	Growth/differentiation factors	Additives to the medium, binding to the artificial matrix	[h, i, j]
	Proliferation versus contact inhibition	Avoiding mitotic stress in serum-free medium	[k, l]
	Long-term culture	Optimizing electrolytes in the medium for human cells	[m]

[a] Minuth et al. (1994)
[b] Sittinger et al. (1994)
[c] Bujia et al. (1995)
[d] Doyle et al. (1993)
[e] Butor et al. (1992)
[f] Taub et al. (1990)
[g] Minuth et al. (1992)
[h] Minuth et al. (1993)
[i] Kloth et al. (1995)
[j] Kloth et al. (1994)
[k] Walsh-Reitz et al. (1984)
[l] Itoh et al. (1994)
[m] Minuth et al. (1995, 1997c)

Fig. 1a–c Tools for tissue engineering. **a** Cell carriers are available in very different forms and functions. The cell carriers consist of a black holder ring and a white spanning ring. In between, an individually selected biomatrix or scaffold with a diameter of 13 or 47 mm is placed. By the biomatrix a plan or three-dimensional proliferation and spreading of the cultures can be triggered. **b** Perfusion culture container for producing artificial tissues. After the cells have adhered to a suitable biomatrix, the cell carriers are transfered into a container for permanent medium perfusion. **c** Gradient culture container for 6 tissue carriers. The tissue carrier is placed with fine forceps in the opened chamber. After closing the chamber, the tissue, for example epithelium, is perfused with different culture media from the upper and lower side just as in vivo

ture containers are connected by silicone tubes and special connectors to form a working line. A peristaltic pump permanently transports fresh culture medium from a bottle to the container, while the used medium is collected in a second bottle. A warming plate is used to keep the temperature at 37°C. As CO_2-independent culture medium is used, the perfusion culture is performed outside an incubator on a laboratory desk for weeks or months.

Epithelia can be colonized just like under natural conditions in between two different compartments. A gradient container with six tissue carriers is used (Fig. 1c). The tissue carriers are placed in the gradient container with forceps and then the cover is closed. The tissue carrier now divides the container into an upper and lower compartment. Very different culture media can be superfused on the luminal and basal side of the epithelia. Thus, individual organo-specific gradient conditions can be simulated (Minuth et al. 1996). If required, the cultures can be kept in the culture containers for weeks and months without subcultivation.

Multiplication of cells

For more than 50 years, methods have been established for isolating cells coming from animal or human tissues (Doyle et al. 1993). Small parts of tissues can be cultivated as an explant, while single cells have to be isolated by proteases. The multiplication of the cells is mostly done in culture dishes with a static medium including growth factors, fetal bovine serum, or autologous serum of a patient (Table1). That way an efficient multiplication is guaranteed and a large number of cell types can be produced in almost any quantity. Examining the cells for their functionality, one can see that the number of cells multiplies quite well, but at the same time they lose many of their typical qualities by dedifferentiation (Koechlin et al. 1991, Minuth et al. 1997b). Isolated chondrocytes, for example, flatten very untypically and look like mesenchymal cells. No basic cartilage substance is built any more. Cultivated parenchymal liver cells do not show any polarization and only fulfill a fraction of their detoxification capacity. Tubular cells of the kidney lose their typical polarity and functionality (Minuth et al. 1997a). Proximal tubular cells lose their brush border and reduce their transporting capacity (Koechlin et al. 1991). It becomes clear that those 'reembryonalized' cells lost many of their typical features but are excellently suited for reproduction and thus for the first phase of tissue engineering.

Starting the differentiation

If implants are intended for clinical indications, the artificial tissues have to show exclusively natural features and must not show altered protein expression if they are not to provoke immunological responses. In contrast to the normal tissue development in vivo, expression patterns in vitro are either different or incomplete. The focus in all tissue engineering methods must therefore be the control of differentiation in cultivated tissues (Tables 1, 2). It must to be taken into consideration that differentiation is controlled at very different cell-biological levels. The expression and conservation of tissue-typical features are fundamentally influenced by the cell's anchorage and thus by the artificial tissue support (Zuk et al. 1989; Butor and Davoust 1992; Minuth et al. 1994). Best results are achieved by seeding the cells on individually chosen materials such as filter supports, fleeces, biodegradable polymers, or sponge-like matrices (Gebelein 1993; Sittinger et al. 1994; Bujia et al. 1995). The cells use the filter surface for two-dimensional growth, while fibers allow a three-dimensional spreading of connective tissues. To improve the cell anchorage, the biomatrices can be coated by synthetic polymers (Woerly et al. 1995, 1996), peptides, or proteins of the extracellular matrix such as polylysine, fibronectin, chondronectin, or laminin (Taub et al. 1990; Aebisher and Bellamkonda 1993; Saltzman et al. 1994; Krewson et al. 1995). The biomatrix can also be coupled with growth factors. As the adhesion of the cells is optimized, fast spreading and directed cell migration can be achieved (Offenhäuser et al. 1995; Bohanon et al. 1996).

Maintaining differentiation

After proliferation of the cells, differentiation has to be induced and maintained. For that purpose, the tissue carriers have to be transferred to culture containers and be permanently perfused with fresh medium (Fig. 1b,c) (Minuth et al. 1992). In contrast to conventional cultures within a static environment, continuous elimination of damaging metabolites along with a permanent renewal of the culture medium is achieved by perfusion. Critical accumulation of any metabolites or paracrine factors is avoided through perfusion and conditions are kept at steady state levels (Sittinger et al. 1996a).

Adjusting the culture medium to the period of the proliferation cycle

A central but still largely disregarded point for the experimental control of differentiation features in artificially produced tissues is the culture medium (Leist et al. 1996; Konstantinov 1996; Gorman et al. 1996). Up to now, all culture media were developed to proliferate the cells as well as possible. For example, it has not been taken into consideration that neural tissue is only able to proliferate during the embryonic phase but is later found in the adult state in a postmitotic phase. The same is true for liver, kidney, or endocrine organs, whose cells are situated in the G_1-phase of the mitotic cycle for years. Consequently, tissue engineering first needs a multiplication phase followed by a differentiation phase. Mitosis and interphase are gradual and not parallel working processes. For this reason tissue engineering has to adapt the culture medium to the mitotic cycle. For proliferation, conven-

tional culture medium with growth factors or fetal calf serum is appropriate. For differentiation, however, growth factors and fetal calf serum have to be reduced and the composition of electrolytes in the culture medium has to be specifically adjusted (Behboo et al. 1994; Kloth et al. 1995; Minuth et al. 1997c).

Adjusting the culture medium to the electrolytes of the serum

Analyses show that the electrolyte composition of culture media does not correspond with the electrolytes found in the serum of animals or humans (Table 3; Minuth et al. 1997c). The electrolyte composition of the culture medium can be very easily analyzed by a Stat profile electrolyte analyzer. For our experiments, we use kidney cells from neonatal rabbits, so the serum composition of those animals will give us a clue to how to compose our culture culture medium if we want to reach differentiation rather than proliferation. Well-known media for cultivation of kidney cells are MEM, DMEM, und IMDM, for example. In the commercially available IMDM 112 mMol/l Na^+, while in serum 137 mMol/l Na^+ was found. It has been proven that the proliferation of cells can be stimulated by increasing the Na^+- or lowering the K^+-concentration (Walsh-Reitz et al. 1984) or by changing the osmolarity (Itoh et al. 1994). This is in the same range as observed for growth factors (Taub et al. 1990). To avoid the permanent mitotic stress on the cells, the single electrolyte parameters are now being adjusted to the electrolyte parameters of the respective serum by adding NaCl or Na^+-gluconate. At the same time, the osmolarity is being optimized in the culture medium. By this method, it was possible to modulate the expression of individual cell features simply by changing the electrolyte parameters (Minuth et al. 1997c). Therefore tissue engineering has to determine experimentally whether media conditions cause cells to divide permanently or to remain in interphase. The typical functions of the tissue that must be maintained in tissue engineering occur only during interphase. Therefore proliferation and differentiation have to be viewed separately and have to be modulated by withdrawing or adding mitogenic stimuli.

The addition of fetal calf serum in many cases causes a dedifferentiation of the tissues (Koechlin et al. 1991). Growth factors or fetal calf serum should therefore only be added to the culture medium during cell proliferation but not during the differentiation phase. To avoid mitotic stress and thus a downregulation of differentiation, adult serum or mostly serum-free medium with added differentiation factors or hormones is used (Minuth et al. 1993; Kloth et al. 1995).

Modulation of differentiation in embryonic tissue

Processes of differentiation proceed naturally in various ways during ontogenesis. However, dedifferentiation of cells under in vitro conditions is so far a mostly unexplored cell-biological phenomenon. It is typical that the individual cells of our organs function perfectly but already lose their typical features during isolation and subsequent culture. Cellular dedifferentiation results in an atypical expression of proteins. This will cause an unwanted immunological reaction after implantation or after blood contact in a biomodule and is completely undesirable for tissue engineering.

Differentiation and dedifferentiation are contrary processes. Basic information about technical and cell-biological possibilities for inducing differentiation and preventing dedifferentiation have been examined in embryonic epithelia of the kidney. Epithelia were kept on tissue carriers in gradient containers (Fig. 1b,c) (Minuth et al. 1992, 1996, 1997a, c). The cells were superfused with a urine-like medium from the upper side and with an interstitial-like culture medium from the lower side as it is found in vivo. Applying this method, it was possible for the first time to generate a defined epithelium from embryonic cells of the collecting duct of the mammalian kidney with its light principal cells and dark intercalated cells. It was possible to maintain the epithelium in its typical phenotype for weeks and months (Aigner et al. 1994). The expression of specific proteins in these epithelia could be drastically influenced by simply adding 12 mMol/l NaCl to the standard culture medium (IMDM, Table 3). On this occasion, it was demonstrated that the development of tissue-typical features does not start immediately after increasing the NaCl-concentration but only with a delay of 5 days. It takes at least 12–14 days for the complete development of typical features (Minuth et al. 1997c). The typical features decreased within 4 days when epithelia were supplied with standard medium after the 14th day. It was further shown that differentiation does not appear automatically, but has to be induced experimentally, for example, by increasing the NaCl-concentration. Here, several factors are working together in an apparently hierarchic system (Table 2). The initial control comes from

Table 3 Analytical parameters of neonatal rabbit serum, standard IMDM, Cl^--, and Na^+-adapted medium for culturing embryonic collecting duct epithelia. Mean±half of range is given (see also Minuth et al. 1997c)

mMol/l	Serum	Standard IMDM	Cl^--adapted IMDM by adding 12 mMol/l NaCl	Cl^- and Na^+-adapted IMDM by adding 12 mMol/l NaCl and 17mMol/l Na-gluconate
Ca^{++}	1.66 ± 0.46	1.15 ± 0.27	1.15 ± 0.1	1.15 ± 0.03
K^+	6.04 ± 1.7	4.25 ± 0.1	4.25 ± 0.04	4.25 ± 0.04
Cl^-	99 ± 5.8	85.1 ± 1.0	98.7 ± 1.4	98.5 ± 1.2
Na^+	137 ± 5.7	112.3 ± 1.6	126.4 ± 1.7	136.9 ± 1.0
	n = 17	n = 40	n = 31	n = 32

the anchorage of the cells in its organo-typical extracellular environment. Extracellular matrix or hormonal factors by themselves cannot effect a complete differentiation (Minuth et al. 1993, 1997b).

In vitro engineering of connective tissues

The typical differentiation has not only a decisive meaning for embryonal, but also for adult tissues. Various successful approaches were undertaken to grow autologous cartilage, bone, and tendon transplants for reconstructive surgery (Sittinger et al. 1994; Bujia et al. 1995; Crane et al. 1995; Sittinger et al. 1996a, b; Wintermantel et al. 1996). For the first time, in 1994, the transplantation of autologous chondrocytes and periostal flaps were described for treatment of traumatic joint defects in humans (Brittberg et al. 1994). Meanwhile, artificial tissue constructs composed of resorbable biomaterials and mesenchymal cells were extensively tested subcutanously in nude mice and in artificial joint defects in chicken and rabbits (Vacanti et al. 1993; Freed et al. 1994; Perka et al. 1996). The medical needs for cartilage repair can be divided into three major areas with quite specific requirements: (1) preshaped cartilage replacement for the plastic surgeon to augment defects, e.g., in the nose or outer ear; (2) highly pressure-resistant hyaline cartilage for the repair of traumatic joint defects; and (3) genetically modified artificial cartilage to treat severe defects due to chronic degenerative cartilage diseases, such as rheumatoid arthritis. In addition to cartilage, there is also an enormous demand for the autologous repair of bone defects such as periprothetic defects, cysts, or fractures.

As an initial step in forming an autologous artificial tissue, an appropriate biopsy has to be collected from the patient. Even for cartilage, which has rather low cell densities compared to other tissues, approximately 20 million cells are required to form one cm^3 of new tissue volume (Sittinger et al. 1996a). Therefore, the possible size of the biopsy and the proliferative capacity of the enzymatically isolated cells are essential factors to be considered. Cell proliferation that precedes the actual tissue engineering step can be effectively carried out in conventional cultures dishes using media supplemented with 10% serum. However, to avoid potential viral or prion contamination, fetal calf serum or any other pooled sera should not be used. Instead, only autologous serum should be chosen wherever it is possible (Gruber et al. 1996).

The main step of tissue engineering resembles an integration of shaping, guiding, or differentiating biomaterials and expanded autologous cells. For the reconstruction of an outer ear, for example, the biomaterial primarily has to provide a temporary scaffold shaping the tissue. Nonwoven fiber structures have been shown to offer a high internal surface for cell attachment and a minimum amount of polymer per volume that has to be resorbed (Sittinger et al. 1994). Currently, mainly polymers of glycolic and lactic acid are applied (Sittinger et al. 1996b; Puelacher et al. 1994). Even though these biomaterials are considered to be very biocompatible, they may affect tissue development due to acidic degradation products in vitro or in vivo and attract cells such as foreign body giant cells after transplantation (Sittinger et al. 1997).

A frequent and major drawback of cell expansion, however, is cell dedifferentiation. Therefore differentiation to a tissue-typic phenotype has to be triggered at a later stage of tissue formation (Sittinger et al. 1996a). After combining cells and scaffold material in vitro, subsequent culture conditions have to support cell differentiation rather than proliferation. For chondrocytes, cell rounding in three-dimensional cultures can trigger redifferentiation and stimulate synthesis of tissue-specific collagen type II (Benya and Shaffer 1982). In addition, morphogenetic factors such as bone morphogenic proteins (BMPs) provide powerful tools in applied tissue differentiation (Reddi 1994; Dudley et al. 1995). Artificial tissue constructs are cultured in perfusion containers under stabilized culture conditions. This is important due to the high nutrient consumption in high density cultures and the release of acidic degradation products from the polymers (Sittinger et al. 1995, 1997). It may further stabilize important autocrine morphogenic signals.

Following transplantation of autologous artificial connective tissues, no major immune responses are expected. However, studies with autologous and xenogenous native cartilage transplants have shown humoral responses to collagen type IX (Buija et al. 1994; Sittinger et al. 1996a; Jerez et al. 1996), which is associated on the surface of collagen fibrils. A possible reason may be a presentation of cartilage matrix epitopes that are normally sequestered from the immune system.

Generation of functional organoids

Besides connective tissues, parenchymal epithelia from different organs are of special interest to tissue engineers. Epithelia are always found at sites in the body where something has to be exchanged or protected. Examples are the protecting skin, the metabolizing parenchymal cells of the liver, and the urine-secreting epithelia of the kidney. As the luminal and basal environment of all epithelia can be clearly distinguished, they function as intelligent biobarriers in a physiological gradient.

If wide areas of a patient's burns have to be covered, it does not matter for the present tissue-engineering procedure whether a single layered or a multilayered tissue with a specific differentiation capacity is at disposal or not. An essential step in saving the patient's life exists first of all in the cultivation of a sufficient area of autologous keratinocytes as quickly as possible in order to be able to cover the burns (von Donnersmarck et al. 1995). However, these autologous transplantates do not form secondary derivates of the skin like sweat and suet glands, and don't form hair roots as well. Therefore it is to be proven by new approaches to what extent not only keratinocytes but real equivalents of the skin can be produced for plastic surgery by tissue engineering.

Tissue engineering experimentally aims at the production of an artificial liver (Gerlach et al. 1994a, b; Jauregui et al. 1996a, b; Naik et al. 1996), pancreas (Mikos et al. 1993; Clayton and London 1996; Deng et al. 1996), or kidney (Cieslinski and Humes 1993). Studies with cultivated liver cells show that parenchymal cells can be kept alive for a long period under in vitro conditions, but they lose the essential function of metabolizing capacity (Gerlach et al. 1995; Bader et al. 1996; Wick et al. 1996). There is no doubt about the fact that the respective cells that are needed can be won by reproduction through the technical possibilities given today. After amplification, the cells have to be transferred from the culture bottle to a technical device in order to function later and be in touch with a corresponding biomatrix, for example, in a capillar bioreactor module (Gerlach et al. 1994a, b; Kono et al. 1995; Gutsche et al. 1996; Lopina et al. 1996). This essential step changes methods discontinuously and can not be calculated as in cell differentiation and later function. Numerous experiments have shown that cells have a good chance of surviving during the transfer from the culturing bottle to a capillary bioreactor module, but essential functions are still downregulated (Gerlach et al. 1996).

A bioreactor based on cultivated parenchymal cells of the liver can be temporarily connected with a patient to bridge comas. Mostly capillary modules are being used (Gerlach et al. 1995). Here, parenchymal cells of the liver are settled in between an artificial capillary meshwork. The capillaries provide the cells with nutrients and oxygen while the patient's blood or serum is passed the bioreactor through an additional meshwork of capillaries. Another possibility is the sandwich-method, where parenchymal cells of the liver are kept in a capillar gap that is built by a biomatrix and two permeable membranes (Roberts et al. 1995; Bader et al. 1996). Although progress has been made in both systems in the last years, the demetabolizing capacity of the liver parenchymal cells in the reactors is not enough to bridge a patient's coma until a suitable liver transplant is found.

Similar problems are caused by implanted pancreatic islet cells. Autologous implants can not be won because of the disease's progression. Therefore not autologous, but heterologous cell material has to be used. To prevent immunological reactions, the heterologous cells are embedded in an artificial extracellular matrix (Ohgawara et al. 1995; Colton 1996; Tatarkiewicz et al. 1996). Then the construct is implanted to produce hormones. However, a downregulation of the insuline production can be observed after a relatively short period (Falorni et al. 1996). A similar process of downregulation and decrease of biotransformation is observed in tissues that are to be implanted in patients who suffer from Morbus Parkinson (Levivier et al. 1995; Date et al. 1996; Kondoh et al. 1996).

As kidney transplants become scarce, the production of an artificial and external working dialysis module based on the patient's own cells has to be considered. The technique used so far is based on a physical filter. Harmful metabolic products pass the pores (Leypoldt and Cheung 1996). A part of these metabolites reaches the body again by rediffusion in the same manner. A dialysis module with cultivated epithelial cells of the kidney would be able to avoid this (Humes 1996a, b). A harmful metabolic product such as urea, for instance, could be transported through the epithelial barrier as through a valve and could not return. In contrast to a conventional membrane, the epithelial cells do not allow the retransport. Experimental work is being performed on a glomerular as well as on a tubular module (Cieslinski and Humes 1993).

Generation of blood vessels

The tissue engineering of blood vessels includes co-cultures of fibroblasts, smooth muscle, and endothelial cells (L'Heureux et al. 1993). First, a three-dimensional but flat meshwork of fibroblasts is used. This meshwork is then formed like a tube and settled with smooth muscle and endothelial cells. The vessel-like construct is perfused with medium for further cultivation. As a matter of principle, the production of these vessel-like constructs works similar to the body's own renewal of vessels. The cellular components for the constructs are won from the patient's own – mostly venous – vessels. After the extraction of a biopsy, the tissue is transferred by proteolytic reduction of the extracellular matrix to a single cell suspension. These cells are then stimulated to proliferate. After several passages when enough cellular material is at hand, the differentiation of the cells is induced. This way vessel-like constructs can be produced that can withstand an internal pressure of no less than 1000 mm Hg. In the future these constructs will be implanted for bypass surgery (L'Heureux et al. 1996).

Stroma and parenchyme

Tissue engineering often builds relatively big or thick pieces of tissue and artificial organoid structures. In multilayers of tissues, it has to be ensured that all of the cells are optimally provided with oxygen and nutrients. Therefore parenchymal cells can be settled in between artificial hollow-fiber capillary nets. Other new possibilities are artificial spreading-vessel systems, which provide the tissue. This can have a special positive effect on the functional features of tissues as an essential interaction is found between parenchymal cells and the stroma (Werb et al. 1996).

The adult organism develops new vessels by angiogenesis (Risau et al. 1988), which means the spreading of endothelial cell cords out of existing vessels. This procedure assumes a complex succession of various reactions. A dissolution of the basement membrane of the vessel has at least partly to take place. Endothelial cells have to be stimulated to proliferate, migrate, and build a luminal space, as found in functional vessels. The generation of vessels is therefore based on a great amount of external signals. Soluble signal substances such as basic fibroblast

growth factor (bFGF) and vascular endothelial growth factor (VEGF), for example (D'Amore und Smith 1993; Moses et al. 1995; Hanahan und Folkman 1996; Mandriota et al. 1996), participate as well as proteases (Pepper et al. 1994; Montesano et al. 1996; Moses et al. 1996) and components of the extracellular matrix do (Gullberg und Ekblom 1995; Furness 1996).

Tissue engineering delivers novel knowledge

Basic know-how of the spreading of vessels in tissues of the kidney could be gained for the first time by perfusion culture (Kloth et al. 1995). For this purpose, pieces of tissue from the neonatal kidney were prepared, clamped to a tissue carrier, and cultured under a continuous perfusion of medium in the presence of angiogenous factors such as bFGF, VEGF, and hormones like aldosterone or 1.25 dihydroxyvitamine D3. The result was an essential expansion of the vessel net. This developmental process is very specific, as it could be stopped by an antibody against VEGF. Besides the know-how about the development of the renal vessel system, the experiments gave new information for the situation in vitro (Kloth et al. 1997). This new information contains the knowledge about the extracellular matrix alone is not a sufficient stimulus for the development of a vessel net that is put in order. Matrix-connected and soluble angiogenous factors work together. Here, the interaction of the different cellular components of the tissue has a more important meaning. Only with the help of such basic information will it be possible to develop an artificial vessel system interacting with the parenchyme of an organ.

Interacting tissue systems

The greater number of previous experiments with cultured cells of varying species gave no sure information about the situation found in vivo. In contrast, interacting artifical tissues made of human cell types will lead to an advanced knowledge and will replace numerous experiments performed on animals (Schulz et al. 1996). What is wanted is that different types of cells communicate through a functioning extracellular matrix. Under realistic conditions, one can analyze the development of diseases. For example, interacting, three-dimensional cultures of synovial cells and chondrocytes were succesfully produced. Along a histologically clearly recognizable tissue-contact zone, local changes can be examined for the first time, such as the phenotype of cells, the degradation of the extracellular matrix, cytokine secretion, or cell invasion. Research on biomaterials and dental medicine are providing another example. In a perfusion culture container (Fig. 1), longterm experiments about medication and toxicity can be performed (Schmalz et al. 1996). Isolated dental slices are used in gradient perfusion containers (Fig. 1c) and mesenchymal cells are grown on the one side of the dental slice. On the other side, materials for treatment and filling of teeth can be applied for almost any period. If the mesenchymal cells do not survive for a defined time, the substance to be tested will not pass the examination. For the first time, in vitro experiments with human cells that previously could only be performed in animal experiments can be carried out by such realistic tissue models.

Vital conservation of tissues

Many operations within the oral cavity require connective tissue and gingiva, which are scarce, to cover the wounds. Here the solution could be a vital conservation of patient's tissue. Gingiva is explanted, for instance, from the inner side of the cheek. The epithelium is subsequently placed in a tissue carrier and kept vital in a perfusion container for weeks (Lehmann et al. 1997). Meanwhile the wound in the oral cavity will heal. For the actual operation, the gingiva conserved in perfusion containers can be applied as additional material to treat periodontal wounds.

Conclusion and future challenge of tissue engineering

Cells and tissues in a healthy organism function perfectly, but under in vitro conditions essential functions are lost. The central question of tissue engineering will be how the experimental control of a tissue-typical cell differentiation can be found and how – at the same time – the atypical dedifferentiation is avoided. Artificial tissue will only be of use for humans if the constructs show the typical functions extracorporally as implants or within a biomodule. The constructs have to develop a perfect functional capacity and cannot be allowed to cause inflammations and immunological rejections compared to conventional implants. Tissue engineering is opening a new market for special institutes that are close to hospitals. Here the patient's own cells can be prepared and tissue engineering can be carried out professionally. To meet all requirements on safety and reliability, it is important to have innovative techniques and products for cultivation as well as a great selection of application-specific artificial matrices to engineer tissues with optimized activities. So far we are usually not able to force the cells to generate functional tissues simply by adding appropriate growth or differentiation factors into a Petri dish. The more practical approach is to find out what kind of environment cells find to be optimal. If we use this knowledge, a functional tissue will automatically develop.

Acknowledgements The project was honored in 1992 with the Philip Morris award 'challenge for the future' and the Hildegard Doerenkamp – Gerhard Zbinden Foundation. Further information about tissue engineering is available from MINUCELLS and MINUTISSUE Vertriebs GmbH, Starenstrasse 2, D-93077 Bad Abbach, Fax: 49 (0)9405 96 24 41; E-mail: minucells.minutissue@t-online.de or on Tissue Engineering Pages: http://www.ukrv.de/ch/rheuma/TissueWeb.html

References

Aebischer P, Bellamkonda R (1993) Review: tissue engineering in the nervous system. Biotech Bioeng 43:543–554

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. Epith Cell Biol 3:70–78

Bader A, Knop E, Kern A, Böker K, Frühauf N, Crome O, Esselmann H, Pape C, Kempka G, Sewing K-F (1996) 3-D coculture of hepatic sinusoidal cells with primary hepatocytes – design of an organotypical model. Exp Cell Res 226:223–233

Behboo R, Ricordi C, Carroll PB, Memarzadeh S, Zeevi A, Conrad B, Rastellini C, Rao AS (1994) Improved long-term culture of functional human islets in serum-free medium. Transplant Proc 26:3301

Benya PD, Shaffer JD (1982) Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype. Cell 30:215–224

Bizios R (1994) Mini-review: osteoblasts: an in vitro model of bone-implant interactions. Biotech Bioeng 43:582–585

Bohanon T, Offenhäuser A, Elender G, Knoll W, Köberle P, Lee J-S, Ringsdorf H, Sackmann E, Simon J, Tovar G, Winnik FM (1996) Neural cell pattern formation on glass and oxidized silicon surfaces modified with poly (*N*-isopropylacrylamide). J Biomat Sci 8:19–39

Boyce ST, Goretsky MJ, Greenhalgh DG, Kagan RJ, Rieman MT, Warden GD (1995) Comparative assessment of cultured skin substitutes and native skin autograft for treatment of full-thickness burns. Ann Surg 222:743–752

Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Eng J Med 331:889–895

Bujia J, Alsalameh S, Naumann A, Wilmes E, Sittinger M, Burmester GR (1994) Humoral immune response against minor collagens type IX and XI in patients suffering from cartilage graft resorption after reconstructive surgery. Ann Rheum Dis 53:229–234

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

Butor C, Davoust J (1992) Apical to basolateral surface area ratio and polarity of MDCK cells grown on different supports. J Exp Cell Res 203:115–127

Cieslinski DA, Humes HD (1993) Tissue engineering of a bioartificial kidney. Biotech Bioeng 43:678–681

Clayton HA, London NJM (1996) Survival and function of islets during culture. Cell Transplant 5:1–12

Colton CK (1996) Engineering challenges in cell-encapsulation technology. Tibtech 14:158–162

Crane GM, Ishaug SL, Mikos AG (1995) Bone tissue engineering. Nat Med 1:1322–1324

D'Amore PA, Smith SR (1993) Growth factor effects on cells of the vascular wall: a survey. Growth Factors 8:61–75

Date I, Ohmoto T, Imaoka T, Ono T, Hammang JP, Francis J, Greco C, Emerich DF (1996) Cografting with polymer encapsulated human nerve growth factor secreting cells and chromaffin cell survival and behavioral recovery in hemiparkinsonian rats. J Neurosurg 84:1006–12

Deng S, Ketchum RJ, Levy MM, Perloff JR, White DJG, Naji A, Brayman KL (1996) Long-term culture or complement inhibition improves early islet function in dog to rat islet xenotransplantation. Transplant Proc 28:805–806

Desgrandchamps F, Tuchschmid Y, Cochand-Priollet B, Therin M, Teillac P, Le-Duc A (1995) Experimental study of Wallstent self-expandable metal stent in ureteral implantation. J Endourol 9:477–81

Donnersmarck GH von, Mühlbauer W, Höfter E, Hartinger A (1995) Die Verwendung von Keratinozytenkulturen in der Schwerbrandverletztenbehandlung – bisherige Erfahrungen, Ausblicke zur weiteren Entwicklung. Unfallchirurg 98:229–232

Doyle A, Griffith JB, Newell DG (1993) Cell and tissue culture. Laboratory procedures. John Wiley & Sons, Chichester

Dudley AT, Lyons KM, Robertson EJ (1995) A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev 7:2795–2807

Falorni A, Basta G, Santeusanio F, Brunetti P, Calafiore R (1996) Culture maintenance of isolated adult porcine pancreatic islets in three-dimensional gel matrices: morphologic and functional results. Pancreas 12:221–229

Frangos JA, Hillsley MV (1993) Review: bone tissue engineering: the role of interstitial fluid flow. Biotech Bioeng 43:573–581

Freed LE, Grande DA, Lingbin Z, Emmanual J, Marquis JC, Langer R (1994) Joint resurfing using allograft chondrocytes and synthetic biodegradable polymer scaffolds. J Biomed Mat Res 28:891–899

Furness PN (1996) Extracellular matrix in the kidney. J Clin Pathol 49:355–359

Gebelein CG (1993) Biotechnological polymers – medical, pharmaceutical and industrial applications. Technomic Publishing, Basel

Gerlach JC, Brombacher J, Klöppel K, Schnoy N, Neuhaus P (1994a) Comparison of four methods for mass hepatocyte isolation from pig and human livers. Transplantation 57:1318–1322

Gerlach JC, Encke J, Hole O, Müller C, Courtney JM, Neuhaus P (1994b) Hepatocyte culture between three dimensionally arranged biomatrix-coated independent artificial capillary systems and sinusoidal endothelial cell co-culture compartments. Int J Artif Org 17:301–306

Gerlach JC, Schnoy N, Encke J, Smith MD, Müller C, Neuhaus P (1995a) Improved hepatocyte in vitro maintenance in a culture model with woven multicompartment capillary systems: electron microscopy studies. Hepatology 22:546–552

Gerlach JC, Fuchs M, Smith MD, Bornemann R, Encke J, Neuhaus P, Riedel E (1996b) Is a clinical application of hybrid liver support systems limited by an initial disorder in cellular amino acid and α-keto acid metabolism, rather than by later gradual loss of primary hepatocyte funcion? Transplantation 62:224–228

Glowacki J, Mizuno S (1996) Chondroinduction of human dermal fibroblasts by demineralized bone in three-dimensional culture. Exp Cell Res 227:89–97

Gorman L, Hennig B, Preston M (1996) Growth requirements of endothelial cells in culture: variations in serum and amino acid concentrations. Nutrition 12:266–270

Gruber R, Sittinger M, Bujia J (1996) Untersuchungen zur in vitro Kultivierung von Humanchondrozyten bei Einsatz FCS-freier Zuchtmedien: Minimierung des möglichen Risikos einer Infektion mit Erregern von Prionen-Erkrankungen. Laryngo-Rhino Otol 75:105–108

Gullberg D, Ekblom P (1995) Extracellular matrix and its receptors during development. Int J Dev Biol 39:845–854

Gutsche AT, Leong KW, Hungnan L, Zurlo J, Yager J (1996) Engineering of a sugar-derivatized porous network for hepatocyte culture. Biomaterials 17:387–393

Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86:353–364

Harvey AR, Woerly S, Plant GW (1996) Cultured rat neuronal and glial cells entrapped within hydrogel polymer matrices: a potential tool for neural tissue replacement. Neurosci Let 205:197–201

Humes H (1996a) The bioartificial renal tubule: prospects to improve supportive care in acute renal failure. Ren Fail 18:405–408

Humes HD (1996b) Application of gene and cell therapies in the tissue engineering of a bioartificial kidney. Int J Artif Org 19:215–217

Itoh T, Yamauchi A, Miyai A, Yokoyama K, KamadaT, Keda N, Fujiwara Y (1994) Mitogen-activated protein kinase and its activator are regulated by hypertonic stress in Madin-Darby Canine Kidney cells. J Clin Invest 93:2387–2392

Jauregui HO, Chowdhury NR, Chowdhury JR (1996a) Use of mammalian liver cells for artificial liver support. Cell Transplant 5:353–367

Jauregui HO, Naik S, Trenkler D, Santangini H, Pan J (1996b) Isolation and culture of porcine hepatocytes for artificial liver support. Cell Transplant 5:107–115

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, Ebenbeck C, Monzer J, Vries U de, Minuth WW (1997) Three dimensional organization of the developing vasculature of the kidney. Cell Tissue Res 287:193–201

Koechlin N, Pisam M, Poujeol P, Tauc M, Rambourg A (1991) Conversion of a rabbit proximal convoluted tubule (PCT) into a cell monolayer: ultrastructural study of cell dedifferentiation and redifferentiation. Eur J Cell Biol 54:224–236

Kondoh T, Pundt LL, Blount JP, Conrad JA, Low WC (1996) Transplantation of human fetal tissue from spontaneous abortions to a rodent model of Parkinson's disease. Cell Transplant 5:69–75

Kono Y, Yang S, Letarte M, Roberts EA (1995) Establishment of a human hepatocyte line derived from primary culture in a collagen gel sandwich culture system. Exp Cell Res 221(2):478–485

Konstantinov KB (1996) Monitoring and control of the physiological state of cell cultures. Biotech Bioeng 52:271–289

Krewson CE, Klarman ML, Saltzman WM (1995) Distribution of nerve growth following direct delivery to brain interstitium. Brain Res 680(1–2):196–206

L'Heureux N, Germain L, Labbe R, Auger FA (1993) In vitro construction of a human blood vessel from cultured vascular cells: a morphologic study. J Vasc Surg 17:499–509

L'Heureux N, Germain L, Labbe R, Auger FA (1996) A tissue engineered human blood vessel (Abstract). J Vasc Res 33:55

Lehmann P, Kloth S, Aigner J, Dammer R, Minuth WW (1997) Lebende Langzeitkonservierung von humaner Gingiva in der Perfusionskultur. Mund Kiefer Gesichtschirurgie 1:26–30

Leist M, Brigelius-Flohé R, Raab B, Maurer S, Rösick U (1996) Conventional cell culture media do not adequately supply cells with antioxidants and thus facilitate peroxide-induced genotoxicity. Free Radic Biol Med 21:297–306

Levivier M, Przedborski S, Bencsics C, Kang UJ (1995) Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. J Neurosci 15:7810–20

Leypoldt JK, Cheung AK (1996) Characterization of molecular transport in artificial kidneys. Artif Org 20:381–389

Lopina ST, Griffith-Cima L, Wu G, Merrill EW (1996) Hepatocyte culture on carbohydrate-modified star polyethylene oxide hydrogels. Biomaterials 17:559–569

Mandriota SJ, Menoud PA, Pepper MS (1996) Transforming growth factor beta 1 down-regulates vascular endothelial growth factor receptor 2/flk-1 expression in vascular endothelial cells. J Biol Chem 271:11500–11505

Mikos AG, Papadaki MG, Kouvroukoglou S, Ishaug SL, Thomson RC (1993) Mini-review: islet transplantation to create a bioartificial pancreas. Biotech Bioeng 43:673–677

Minuth WW, Dermietzel R, Kloth S, Hennerkes B (1992) 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, Stöckel G, Dermietzel R (1993a) Aldosterone modulates PNA binding cell isoforms within renal collecting duct epithelium. Kidney Int 44:537–544

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

Minuth WW, Kloth S, Aigner J, Steiner P (1995) MINUSHEET-Perfusionskultur: Simulierung eines gewebetypischen Milieus. Bioscope 4:20–25

Minuth WW, Aigner J, Kloth S, Steiner P, Tauc M, Jennings ML (1997) Culture of embryonic renal collecting duct epithelia in a gradient container. Pediatr Nephrol 163:140–147

Minuth WW, Aigner J, Kubat B, Kloth S (1997b) Improved differentiation of renal tubular epithelium in vitro: potential for tissue engineering. Exp Nephrol 5:10–17

Minuth WW, Steiner P, Strehl R, Kloth S, Tauc M (1997c) Electrolyte environment modulates differentiation in embryonic renal collecting duct epithelia. Exp Nephrol (in press)

Montesano R, Kumar S, Orci L, Pepper MS (1996) Synergistic effect of hyaluronan oligosaccharides and vascular endothelial growth factor on angiogenesis in vitro. Lab Invest 75:249–62

Moses MA, Klagsbrun M, Shing Y (1995) The role of growth factors in vascular cell development and differentiation. Int Rev Cytol 161:1–48

Moses MA, Marikovsky M, Harper JW, Vogt P, Eriksson E, Klagsbrun M, Langer R (1996) Temporal study of the activity of matrix metalloproteinases and their endogenous inhibitors during wound healing. J Cell Biochem 60:379–86

Naik S, Trenkler D, Santangini H, Pan J, Jauregui HO (1996) Isolation and culture of porcine hepatocytes for artificial liver support. Cell Transplant 5(1):107–115

Nerem RM, Sambanis A (1995) Tissue engineering: from biology to biological substitutes. Tissue Eng 1:3–11

Offenhäuser A, Rühe J, Knoll W (1995) Neuronal cells cultured on modified microelectronic device surfaces. J Vasc Sci Technol A 13:2606–2612

Ohgawara H, Miyazaki J-I, Karibe S, Tashiro F, Akaike T, Hashimoto Y (1995) Embedded-culture of pancreatic β-cells derived from transgenic mouse insulinoma as a potential source for xenotransplantation using a diffusion chamber. Cell Transplant 4:307–313

Oliva A, Ragione FD, Salerno A, Riccio V, Tartaro G, Cozzolino A, Amato SD, Pontoni G, Zappia V (1996) Biocompatibility studies on glass ionomer cements by primary cultures of human osteoblasts. Biomaterials 17:1351–1356

Pepper MS, Vassalli JD, Wilks JW, Schweigerer L, Orci L, Montesano R (1994) Modulation of bovine microvascular endothelial cell proteolytic properties by inhibitors of angiogenesis. J Cell Biochem 55:419–434

Perka C, Lindenhayn K, Heilmann HH, Sittinger M, Muschik M (1996) Experimentelle Untersuchungen mechanisch induzierter Gelenkknorpeldefekte nach Implantation allogener embryonaler Chondrozyten in einem Kollagen-Fibrin-Gel beim Huhn. Z Orthop 134:562–571

Puelacher WC, Mooney D, Langer R, Upton J, Vacanti JP, Vacanti CA (1994) Design of nasoseptal cartilage replacements synthesized from biodegradable polymers and chondrocytes. Biomaterials 15:774–778

Reddi AH (1994) Symbiosis of biotechnology and biomaterials: applications in tissue engineering of bone and cartilage. J Cell Biochem 56:192–5

Rennekampff HO, Kiessig V, Hansbrough JF (1996) Current concepts in the development of cultured skin replacements. J Surg Res 62:288–295

Risan W, Sarida H, Zerwes HG, Sasse J, Ekblom P, Kemler R, Doetschman T (1988) Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. Development 102(3):471–478

Roberts EA, Kono Y, Yang S, Letarte M (1995) Establishment of a human hepatocyte line derived from primary culture in a collagen gel sandwich culture system. Exp Cell Res 221:478–485

Saltzman WM, Krewson CE, Chung SW, Dai W (1994) Cell aggregation and neurite growth in gels of extracellular matrix molecules. Biotech Bioeng 43:555–562

Schmalz G, Garhammer P, Schweiki H (1996) A commercially available cell culture device modified for dentin barrier tests. J Endodontics 21:249–252

Schmalzried TP, Peters PC, Maurer BT, Bragdon CR, Harris WH (1996) Long-duration metal-on-metal total hip arthroplasties with low wear of the articulating surfaces. J Arthroplasty 11:438–44

Schulz O, Keyszer G, Sittinger M, Burmester GR (1997) Development of in vitro model systems for destructive joint diseases. Novel strategies to establish inflammmatory pannus. Arthritis Rheum (in press)

Service RF (1995) Designer tissues take hold. Science 270:230–232

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

Sittinger M, Schulz O, Golla J, Keyßer G, Burmester GR (1995b) Artificial cartilage: in vitro model for destructive joint diseases. XXII Congress European Society for Artificial Organs, Berlin. J Artif Organs 18:470

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

Sittinger M, Reitzel D, Dauner M, Hierlemann H, Kastenbauer E, Hammer C, Planck H, Burmester GR, Bujia J (1996b) Resorbable polyesters in cartilage engineering: affinity and biocompatibility of polymer fiber structures to chondrocytes. J Biomed Mater Res 33:57–63

Sittinger M, Schultz O, Keyszer G, Minuth WW, Burmester GR (1997) Artificial tissues in perfusion culture. Int J Artificial Organs 20(1):57–62

Tatarkiewicz K, Sitarek E, Sabat M, Orlowski T (1996) Long-term culture of non-purified rat islets embedded in hydrogel matrix. Transplant Proc 28:831–832

Taub M, Wang Y, Szczesny TM, Kleinmann HK (1990) Epidermal growth factor or transforming growth factor α is required for kidney tubulogenesis in matigel cultures in serum-free medium. Proc Natl Acad Sci USA 87:4002–4006

Vacanti CA, Kim W, Upton J, Vacanti MP, Mooney D, Schloo B, Vacanti JP (1993) Tissue-engineered growth of bone and cartilage. Transplant Proc 25:1019–1021

Walsh-Reitz MM, Aithal HN, Toback FG (1984) Na regulates growth of kidney epithelial cells induced by lowering extracellular K concentrations. Am J Physiol 247:C321–C326

Werb Z, Sympson CJ, Alexander CM, Thomasset N, Lund LR, MacAuley A, Ashkenas J, Bissell MJ (1996) Extracellular matrix remodeling and the regulation of epithelial-stromal interactions during differentiation and involution. Kidney Int 49:68–74

Wick M, Koebe HG, Schildberg FW (1996) Extracorporal artificial liver: the influence of a second cell layer on the morphology and function of immobilized human hepatocytes. Int J Artif Organs 19:415–421

Wilczek K, Scheerder ID, Wang K, Verbaken E, Piessens J (1996) Comparison of self expanding polyethylene terephthalate and metallic stents implanted in porcine iliac arteries. Cardiovasc Intervent Radiol 19:176–80

Wintermantel E, Mayer J, Blum J, Eckert KL, Luscher P, Mathey M (1996) Tissue engineering scaffolds using superstructures. Biomaterials 17:83–91

Woerly S, Laroche G, Marchand R, Pato J, Subr-V, Ulbrich K (1995) Intracerebral implantation of hydrogel-coupled adhesion peptides:tissue reaction. J Neural Transplant Plast 5(4):245–255

Woerly S, Plant GW, Harvey AR (1996) Neural tissue engineering: from polymer to biohybrid organs. Biomaterials 17:301–310

Zuk A, Matlin KS, Hay ED (1989) Type I collagen gel induces Madin-Darby canine kidney cells to become fusiform in shape and lose apical-basal polarity. J Cell Biol 108:903–919