# Tissue Engineering in Otorhinolaryngology

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## ABSTRACT

**Tissue engineering is a field of research with interdisciplinary cooperation between clinicians, cell biologists, and materials research scientists. Many medical specialties apply tissue engineering techniques for the development of artificial replacement tissue. Stages of development extend from basic research and preclinical studies to clinical application. Despite numerous established tissue replacement methods in otorhinolaryngology, head and neck surgery, tissue engineering techniques opens up new ways for cell and tissue repair in this medical field. Autologous cartilage still remains the gold standard in plastic reconstructive surgery of the nose and external ear. The limited amount of patient cartilage obtainable for reconstructive head and neck surgery have rendered cartilage one of the most important targets for tissue engineering in head and neck surgery. Although successful *in vitro* generation of bioartificial cartilage is possible today, these transplants are affected by resorption after implantation into the patient. Replacement of bone in the facial or cranial region may be necessary after tumor resections, traumas, inflammations or in cases of malformations. Tissue engineering of bone could combine the advantages of autologous bone grafts with a minimal requirement for second interventions. Three different approaches are currently available for treating bone defects with the aid of tissue engineering: (1) matrix-based therapy, (2) factor-based therapy, and (3) cell-based therapy. All three treatment strategies can be used either alone or in combination for reconstruction or regeneration of bone. The use of respiratory epithelium generated *in vitro* is mainly indicated in reconstructive surgery of the trachea and larynx. Bioartificial respiratory epithelium could be used for functionalizing tracheal prostheses as well as direct epithelial coverage for scar prophylaxis after laser surgery of shorter stenoses. Before clincal application animal experiments have to prove feasability and safety of the different experimental protocols. All diseases accompanied by permanently reduced salivation are possible treatment targets for tissue engineering. Radiogenic xerostomia after radiotherapy of malignant head and neck tumors is of particular importance here due to the high number of affected patients. The number of new diseases is estimated to be over 500,000 cases worldwide. Causal treatment options for radiation-induced salivary gland damage are not yet available; thus, various study groups are currently investigating whether cell therapy concepts can be developed with tissue engineering methods. Tissue engineering opens up new ways to generate vital and functional transplants. Various basic problems have still to be solved before clinically applying *in vitro* fabricated tissue. Only a fraction of all somatic organ-specific cell types can be grown in sufficient amounts *in vitro*. The inadequate *in vitro* oxygen and nutrition supply is another limiting factor for the fabrication of complex tissues or organ systems. Tissue survival is doubtful after implantation, if its supply is not ensured by a capillary network.**

## INTRODUCTION

**T**HE REPLACEMENT OR RESTORATION of cell, tissue, and organ functions is among the most important challenges of modern medicine. New treatment approaches like organ transplantation or implant management can now improve the quality of life and prolong survival in many patients.

Despite advances in transplantation medicine, the survival of patients with terminal organ failure is jeopardized, particularly by the lack of suitable donor organs. In the mid-nineties, for

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example, about 39,000 people were waiting for a kidney in all of Europe, more than 9,500 in Germany alone (109). In the meantime, expensive replacement therapies like dialysis are required in cases of kidney failure. Moreover, life-long immunosuppression still remains indispensable after organ transplantation, and may involve such complications as therapy-resistant infections and even the development of malignant tumors. Rejection reactions are common despite modern immunosuppressives (51).

Implants have thus far not achieved the quality, longevity, or functionality of natural body parts. Since they are foreign bodies in the organism, material-specific compatibility problems can adversely affect their clinical application. Inserting an implant in an organism always triggers a tissue reaction. Ideally, it hardly differs from the processes in normal wound healing. An unfavorable course may involve the formation of a hard connective-tissue capsule around the implant or a chronic inflammation (11). This could result in an implant loss necessitating further reconstructive measures.

An alternative to human transplants is now available in the form of animal cells, tissues, and organs, one example being the use of porcine hepatocytes in bridging systems for liver replacement (32,57,82). However, xenografting as an interspecies transplantation of cells, tissues and organs is currently no alternative for replacing of human tissue. It involves incalculable risks like the transmission of porcine endogenous retroviruses (PERV) associated with the use of porcine transplants. Like any other retrovirus, PERV can also introduce genes into the host genotype and thus damage the human organism in a hitherto unknown manner (113).

The patient's own tissue is ideal for replacement in view of its immunological safety. However, it is of limited availability and usually insufficient for repairing larger defects. This problem would be solved by generating the patient's own tissue in the therapeutically required amount and quality. Researchers have worked for decades on cultivating and generating human cells and tissue outside the human organism (44). At the beginning of the last century, Ljuggren was first to succeed in keeping human pieces of skin alive in physiological medium for several weeks and replanting them. Growing cells from tissue explants *in vitro* was pursued as a possible approach for tissue cultivation in the sixties. A substantial increase of the tissue surface could not be achieved, however (162). Improved cell culturing techniques first achieved a proliferation of skin cells over several generations. In 1975, Rheinwald and Green described the successfully grown skin cells as a well-differentiated multilayered cell clone that could be cultivated over 20 to 50 generations (147). These advances led to the first therapeutic application of *in vitro* generated skin grafts for severe burn injury victims in the early eighties (8,31). *In vitro*-generated skin grafts have since become the sole means for survival of life-threatening burns worldwide (43,144).

Tissue Engineering is the bonding of laboratory-generated tissues, cells and molecules to one or more biomaterials to correct a defect or compensate for a functional loss in an organism (98).

Since the mid-eighties, tissue engineering has been an independent field of research with interdisciplinary cooperation between clinicians, cell biologists, and materials research scientists. Current concepts for generating bioartificial tissue are decidedly manifold. Most require proliferative human cells that are grown after isolation by suitable cell culturing techniques until they are numerous enough for seeding onto a matrix structure. These scaffold materials are constructed largely as porous networks or sponges of collagen, fibrin, or other components of the natural extracellular matrix (ECM). However, synthetics like polytetrafluoroethylene (PTFE) or polyethylene terephthalate (PET) are also used for making cell and tissue scaffolds (187). Most tissue engineering concepts aim at resorption of the scaffold material by the cells and their metabolites to avoid the long-term disadvantages associated with implants.

Many medical specialties apply tissue engineering techniques for the development of artificial replacement tissue. Stages of development extend from basic research (e.g., pancreas) and preclinical studies (e.g., cardiac valves) to clinical application (e.g., skin and cartilage). Despite numerous established tissue replacement methods, new concepts based on tissue engineering techniques must be developed in otolaryngology, head and neck surgery.

## CELL AND TISSUE CULTURE

The successful propagation of human cells and tissue *in vitro*, the control of their differentiation, and the maintenance of specific functions are the decisive prerequisites for generating clinically relevant transplants with the aid of tissue engineering (117).

### Culture flasks

Basically all cells and tissue should be cultured in disposable vessels for tissue engineering. The advantage is that patient-related systems are already used from the start when developing new replacement tissue, and the standards of "good manufacturing practice" are met. The currently applied flasks, dishes, and tubes consist largely of specially pretreated polysterol. The manufacturer modifies the normally hydrophobic surface of these vessels by gamma irradiation, chemical methods, or arc treatment, possibly applying the plasma procedure in a vacuum. Pretreatment of culture flasks with certain substances such as polylysine, fibronectin, collagen, or gelatin makes it possible to improve the tissue-specific conditions *in vitro*. In tissue engineering, this type of culture flask preparation is usually of secondary importance except for cell proliferation, since here the scaffold structures are used as a substrate for cells and tissue.

### Culture media

Cells and tissues removed from an organism require *in vitro* conditions conducive to proliferation, differentiation, and typical cell functions. Thus, the cells and tissues must be supplied with all substances they cannot synthesize themselves. Cell culture media are mostly composed of amino acids, salts, vitamins, and other additives. All harmful cell products must be neutralized *in vitro* as long as possible by appropriate buffers like $NaHCO_3$. The osmolarity and electrolyte concentration decisively influence the differentiation of cultured tissue (75,116). These parameters should therefore be individually adjusted to the cultured tissue. Antibiotics and antimycotics can be added

for prophylaxis against infections to compensate for the lack of an immune system *in vitro*.

The addition of serum, for example, fetal calf serum (FCS), or growth factors is usually necessary for the proliferation of cells. At the same time, however, these mitogenic stimuli lead to dedifferentiation of cells and tissues *in vitro* (92,115). To achieve differentiation of proliferated cells for tissue engineering, FCS, and growth factors should be reduced in the medium or replaced by suitable differentiation factors (117). Serum additives of animal origin also have other disadvantages: FCS is extracted from the blood of bovine fetuses by centrifugation. Sterilization by filtration can remove mycoplasmas from the serum, but the endotoxin content remains unaffected (103). Moreover, the risk estimation for bovine products is still incomplete. Thus, there is a potential risk of infection with pathogens of prion diseases like the new variant of the Creutzfeldt-Jacob syndrome. A possible solution may be the application of serum-free media or the patient's own serum (60).

### Perfusion cultures and bioreactors

A capillary system constantly supplies nutrients to the tissue *in vivo*. Conventional culture systems are an inadequate substitute for these physiological conditions. This results in an at least partial loss of cell- and tissue-specific functions (90,91). To solve this problem, perfusion culture systems were developed that ensure a constant supply of fresh nutrient solution and concomitant drainage of the stale culture medium and noxious metabolites (Fig. 1) (114,118). The cells or tissues are placed on tissue scaffolds (yellow arrow), and can then be put into the perfusion container. The tissue scaffold forms a barrier between the upper and lower perfusion space so that gradients can be generated by applying various media. Physiological conditions can thus be copied *in vitro*, particularly for epithelial cultures. The medium is oxygenated by diffusion via the walls of the relatively long silicone tubes.

Rotating bioreactors were developed to investigate *in vitro* conditions in a state of weightlessness (137,157). The rotation speed of the culture flasks can be adjusted so that the cultivated material is in a constant state of free fall. These systems were tested for tissue engineering or cartilage with the idea that, under these conditions, it may be possible to achieve definable fluid dynamics with a more efficient supply of nutrients and gases to the cultivated cell–polymer construct (52,53). Suitable rotation modules were used as culture flasks for growing cartilage in the Russian space station "Mir" to investigate the real influence of weightlessness during *in vitro* generation of new tissue. The cartilage constructs cultivated in "Mir" were smaller and had poorer biomechanical properties than the control group cultivated in the same reactor system on Earth (54,55).

## BIOMATERIALS AND SCAFFOLDS

All materials used to make scaffolds for tissue engineering must be classified as biomaterials. They are by definition nonvital materials selected for interaction with a biological system (186). Biomaterials can be modified by suitable procedures to generate cell or tissue scaffolds for tissue engineering. The different steps in the process usually relate to the form, the mechanical properties, and the surface structure of the material, and aim at providing highly physiological guiding structures for cell ingrowth. The architecture of the scaffold material is defined by certain structural elements like pores, fibers, or membranes. These can be constructed according to stochastic, fractal, or periodic principles (187,189). Complex scaffold structures can be constructed by a combination of simple subsystems. Flat two-dimensional cell scaffolds can be transformed into a three-dimensional construct by coiling, for example. Several such units can be combined to form superstructures (188).
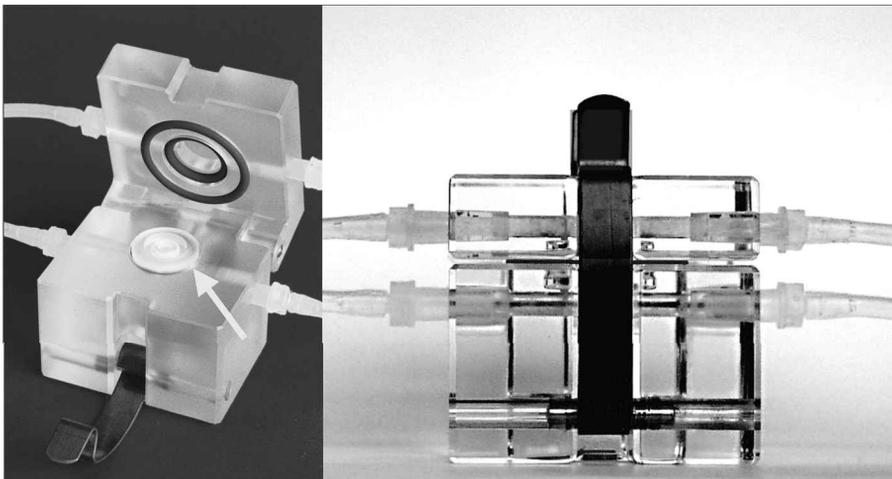


**FIG. 1.**   Open perfusion chamber with inserted tissue scaffold (arrow). The perfusion chamber shown can also be used as a gradient culture system. The cells or tissues cultivated on the tissue scaffold can be perfused from both a luminal (dark medium) and a basal (clear medium) direction (with permission of Professor W. W. Minuth, Institute for Anatomy, University of Regensburg, Germany).

Many different biomaterials are now used to generate cell and tissue scaffolds for tissue engineering. The most frequent are biodegradable polymers in the form of nets, fleeces, or foams of polyglycolic acid, or polyactide and natural polymers like collagen or hyaluronic acid. Ceramic materials like hydroxyapatite are used primarily for tissue engineering of bone (178).

*Biodegradation*

Most approaches for generating new tissue *in vitro* are currently based on the concept of cultivating the patient's own cells on biodegradable and absorbable scaffolds in the form of the organ to be replaced. Precise knowledge of the mechanisms involved in the degradation of the cell scaffold is of decisive importance for tissue engineering, since resultant degradation products or pH changes can drastically impair cell vitality and proliferation (106).

Biodegradable polymers can be broken down by various mechanisms (187): mechanical influences, temperature, or radiation induce degradation by splitting covalent bonds. The resultant free radicals cause further disintegration of the polymer chains.

Hydrolysis leads to degradation of polymers with hydrolytically unstable bonds like ester or amide groups. As a rule, the hydrolytic degradation is controlled by diffusion of $H_2O$; the degradation kinetics are in first approximation independent of the exposed surface of the polymer. Acids and bases as well as enzymes and temperature changes can act as catalyzers of hydrolytic degradation. Aliphatic polyesters are relatively sensitive to hydrolysis. Polyester urethanes can, for example, be degraded in physiological surroundings by hydrolysis of the aliphatic polyester segments. In contrast, aromatic polyesters like PET react less sensitively to hydrolytic influences, since their aromatic groups lend them greater hydrophobicity.

As a rule, enzymatic degradation requires specific groups of molecules that can be recognized by the enzyme. Depending on the enzyme, degradation takes place by hydrolysis, oxidation, or direct chain cleavage. Since the enzymes cannot diffuse into the polymer due to their high molecular weight, the degradation kinetics of enzymatically degraded polymers are determined by the surface accessible to adsorption.

*Generating and modifying scaffold structures*

Three-dimensional cell and tissue scaffolds can now be constructed by various engineering techniques like crosslinking of fibers or leaching (105). As in implant designing, rapid prototyping can be used to generate individual cell and tissue scaffolds from different biomaterials (50,183). The data needed for the three-dimensional scaffold geometry are imported from CT or MRI data sets and converted to production data by system-specific software. These data then control a 3D dosing system, a so-called bioplotter, which doses a material into a different medium. The tip spatially positions and connects drops or microfilaments of polymer solutions, pastes, dispersions and aqueous systems like cell suspensions or hydrogels. The generation of scaffold material by rapid prototyping can one day be integrated into the cell culture process through the further development of bioreactors.

The surfaces of scaffold materials can be modified as in the preparation of culture flasks. The degree of on- or ingrowth of

cells and tissues can be influenced by reactive chemical groups or ligands. For example, the amount of protein bound to the scaffold surface is responsible for the accumulation of further adhesion molecules, especially integrins. Since the expression of various integrin subtypes is cell type-specific (74), the attachment behavior of a particular cell population can be controlled by selection of the protein coating.

# CARTILAGE

Autologous cartilage still remains the gold standard in plastic reconstructive surgery of the nose and external ear (133). The cartilage is usually taken from the rib, the auricle, or the nasal septum (121,145). As a rule, this requires a second session, which may lead to additional complications like wound infections, an unsatisfactory cosmetic result at the donor site, and postoperative pain. The removal of rib cartilage involves the additional risk of pneumothorax as well as possible changes in the thorax contour with subsequent functional disorders (126,170).

The limited amount of patient cartilage obtainable for reconstructive head and neck surgery and the much more frequent indications for cartilage replacement in orthopedic surgery, traumatology, and rheumatology have rendered cartilage one of the most important targets for tissue engineering.

Cartilage is an avascular tissue composed of chondrocytes embedded in an extracellular matrix of collagens, proteoglycans, and noncollagenous proteins. In the head and neck region, cartilage serves primarily as a supporting structure. Mechanical loads like compression, traction, or torsion play a subordinate role compared to articular cartilage.

Cartilage has a low regeneration potential due to the lack of perfusion and the limited number of chondrocytes in the extracellular matrix (ECM), which also evidence low mitotic activity (41,58).

Most strategies for tissue engineering of cartilage are based on the use of biodegradable polymers as temporary scaffold materials for differentiated chondrocytes or precursor cells (175). The removed cells are grown in culture, applied to the scaffold material *in vitro* and then transplanted. The differentiated cells should then produce their tissue-specific matrix components *in vivo*. The tissue thus obtained should resemble native cartilage as closely as possible in its morphological and functional properties.

*Cultivating human chondrocytes*

Cartilage is obtained from septal grafts for most tissue engineering approaches in otolaryngology, head and neck surgery (64,150,160). Removal of the connective tissue and perichondrium is followed by mechanical mincing—with a scalpel, for example—into approx. $1 \times 1$-mm pieces of cartilage. These are incubated for 12 to 18 h in an enzyme solution consisting of a mixture of various enzymes that digest cartilage matrix (type II collagenase, hyaluronidase, etc.). After further purification steps, the chondrocytes exist in the form of a cell suspension. Sittinger *et al.* was able to isolate about $0.5 \times 10^6$ cells with more than 85% vitality from 0.5 cm³ cartilage specimens and achieve a 100 to 100,000 times higher cell count *in vitro* within

8 weeks (161). For this purpose, the chondrocytes were cultivated in DMEM with an admixture of FCS. This medium had already been used by Bruckner *et al.* as well as by Elima and Vuorio (17,48). Other study groups describe the use of Ham's F12 medium (95,169) or RPMI (27) for cultivating chondrocytes.

Strong proliferation of chondrocytes leads to a loss of their tissue-specific features, dedifferentiation, after a few days (182). Cells must have their typical characteristics, however, for *in vitro* generation of functional tissue. Knowledge about controlling the proliferation and differentiation of human chondrocytes *in vitro* is therefore of decisive importance for tissue engineering.

### Proliferation and differentiation of chondrocytes in vitro

Through endochondral ossification, mesenchymal precursor cells that synthesize type I and II collagen develop *in vivo* into chondrocytes that largely form type II collagen after complete differentiation. They initially produce type II and $\times$ collagens in a hypertrophic state, then type I and $\times$ collagens after the termination of hypertrophy (Fig. 2).

Similar processes are also observed *in vitro*. In monolayer cultures, chondrocytes rapidly take on an elongated fibroblastoid shape with cell processes in which actin filaments of the cytoskeleton form stress fibers (110). The cartilage-specific synthesis of type II collagen is stopped (182), and the chondrocytes produce type I and III collagens instead (4,33,111). The chondrocytes start to reproduce at this dedifferentiated stage. Their proliferative activity can be increased by adding certain medium components like fetal calf serum (see earlier) or suitable growth factors (see next section).

Redifferentiation of cells is possible under culture conditions that permit three-dimensional chondrocyte grouping, as, for example, in an agarose gel. They regain their round shape and again synthesize type II collagen and proteoglycans (4,9). The number of differentiated chondrocytes *in vitro* is also influenced by the primary cell count in the monolayer via autocrine stimulation mechanisms (76).

### Influence of growth factors and hormones

Chondrocytes and their precursor cells are subject to various regulatory influences. Growth factors are important for maintaining cartilage in the human organism under different functional conditions (171). Insulin-like growth factor stimulates chondrocyte proliferation *in vitro* and *in vivo* and increases the synthesis of the ECM (128,136). Transforming growth factor beta (TGF-$\beta$) is a multifunctional protein that regulates many cell properties in a time- and dose-dependent manner, especially proliferation, differentiation, and metabolism of the ECM (124). In monolayer cultures TGF-$\beta$ can both increase and inhibit the synthesis of type II collagen (73,96,148,180). In 3D cultures of bovine chondrocytes, the addition of TGF-$\beta$ enhances the development of cartilaginous tissue with an increased content of type II collagen and sulfated glycosaminoglycans (194). Fibroblast growth factor (FGF) is a strong mitogenic stimulus for chondrocytes in joint cartilage, where it regulates both anabolic and catabolic functions (88,130,153). FGF induces the differentiation of mesenchymal stem cells to chondrocytes with increased production of type II collagen and proteoglycans in full-layer cartilage defects (56). Pharmacologically dosed insulin stimulates the proliferation and proteogycan synthesis of chondrocytes (164,195).

### Extracellular matrix and scaffold structures

One of the most important prerequisites for *in vitro* generation of cartilage tissue is the redifferentiation of proliferated chondrocytes by adequate spatial orientation of the cells. This is mediated primarily by the 3D shape and the porosity of the scaffold structure. The experimentally determined porosity of cartilage is 78% (3,19). Highly porous scaffold materials imitate these conditions and, through a high surface–volume ratio, ensure maximal adhesion and proliferation of the seeded chondrocytes and provide adequate space for the synthesized ECM (2). Since the cartilage lacks vascularization, its nutrient supply and metabolism are maintained by diffusion processes. Thus, the scaffold structures must not be diffusion barriers. This is particularly important when cultivating large numbers of cells for the generation of clinically relevant cartilage grafts.

The mechanical properties of the scaffold material should be able to withstand the physiological forces at the implantation site until normal cartilage forms. Native cartilage has a compression modulus of about 0.79 Mpa and a shearing modulus of 0.68 MPa (3,119). Recent studies have shown that the biomechanical properties of *in vitro* generated cartilage grafts can correspond to these strength factors (45).
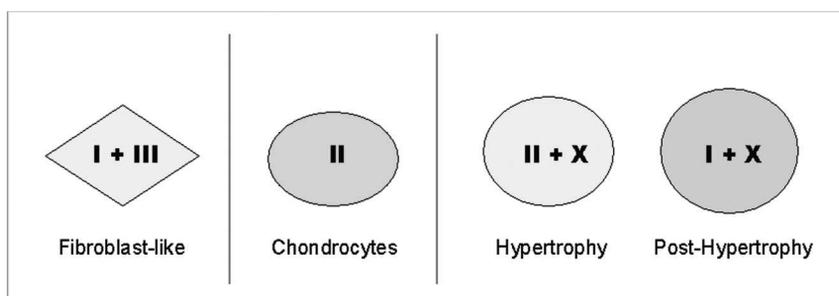


**FIG. 2.** Depiction of the differentiation of chondrocytes *in vitro* [modified according to (29)].

In vitro *generated cartilage grafts*

3-D cartilage constructs have thus far been fabricated primarily by chondrocytes proliferating on biodegradable polymers. The Boston study group of Vacanti and Puelacher reported the first relevant results for the head and neck reason in 1992 and 1994. They had succeeded in fabricating shaped cartilage constructs for the auricle (176) and trachea (177) as well as for the mandibular joint and nasal septum (139,140). The initial material comprised chondrocytes isolated from bovine articular cartilage. However, Rotter *et al.* and Haisch *et al.* were able to show that these results can also be obtained with human cells (64,149). All these approaches involve seeding chondrocytes onto a scaffold *in vitro* and transplanting it into an athymic nude mouse model so that the cell–polymer construct can develop into normal cartilage.

Autologous chondrocyte implantation (16) has now been approved by the Federal Drug Administration for treating articular cartilage defects (13). In otolaryngology, head and neck surgery, treatment with chondrocyte suspensions is less important than implantation of custom-shaped grafts. At present, the clinical application of cartilage grafts fabricated *in vitro* is still restricted to individual cases.

In 1997, the Department of Otorhinolaryngology of the Charité Hospital, Berlin, Germany, made the first attempt to treat a patient with an auricle fabricated *in vitro* (Fig. 3). However, the cosmetic result was not satisfactory, since the bioartificial cartilage had a stable shape for only 3 weeks and was then partially resorbed (66). In 2000, a study on reconstructing a traumatic auricular defect with cartilage generated *in vitro* originated from the Department of Plastic and Hand Surgery at the University of Freiburg in Germany (134). Questions about the postoperative outcome have yet to be answered.

Thus, it has apparently not yet been possible to adequately protect the new cartilage in its graft bed against rejection reactions and resorption processes. This is attributed not only to the macrophage-mediated cellular reactions associated with physiological wound healing but also to antibodies against different collagen types (28,123). One way of protecting the cartilage graft against resorption may be to encapsulate the tissue to provide an adequate immune barrier. A suitable method was described by Lim and Sun for microencapsulation of pancreas tissue. The alginate polylysine method enabled successful encapsulation of Langerhans islet cells and prevented unwanted immunoreactions (102). The alginate extracted from sea algae is unsuitable for encapsulating cartilage because of its mechanical properties and possible toxic by-products (65). In 1996, Dautzenberg *et al.* described a method for tissue encapsulation with sodium cellulosesulphate (NaCs) and polydiallyldimethylammonium chloride (PDADMAC). Both substances spontaneously form capsule membranes that have been found to exhibit mechanical stress resistance and biocompatibility under physiological conditions (38,39). This microencapsulation was first tested in native human cartilage grafts by Haisch *et al.*, and investigated in an animal model. Encapsulated and unencapsulated pieces of cartilage were implanted in athymic nude mice for up to 16 weeks. The histological workup disclosed progressive resorption of the chondrocytes and cartilage matrix. Scattered calcification of the cartilage ground substance and

chondrocyte necroses were also detected as signs of progressive cartilage degeneration. Connective tissue filaments were seen after 12 weeks with vessels, granulocytes, and macrophages proliferating far into the central regions of the cartilage grafts. Encapsulated cartilage, on the other hand, only showed a more marked inflammatory reaction at the capsule membrane (Fig. 4). Resorptive reactions could be detected only where the polyelectrolyte membrane was torn. Thus, encapsulation with NaCs/PDADMAC membranes is a promising solution to the problem of postoperative resorption of *in vitro*-fabricated cartilage grafts.

# BONE

Replacement of bone in the facial or cranial region may be necessary after tumor resections, traumas, inflammations, or in cases of malformations. The ideal replacement material should be mechanically stable to ensure adequate protection of the brain, for example, directly after implantation. A light weight and low heat conduction reduce the risk of pressure- or heat-dependent damage to surrounding tissue. The replacement material can ideally be molded intraoperatively, and easily fixed by osteosynthesis. For postoperative imaging diagnostics, implants should be radiolucent and nonmagnetic for the MRI. Since few replacement materials have met these demands thus far, autologous bone grafts from the outer table or iliac crest, for example, rank high in the reconstruction of bony defects of the facial skull and cranium (154). However, autologous bone grafts usually require a second intervention that can cause cosmetically unfavorable scars or recurrent pain (71). Removal of bone grafts from the skull cap may involve seromas, hematomas, wound-healing impairments, dura lesions, subarachnoid bleeding, and opening of the sagittal sinus, as well as intracerebral hemorrhages (138,156). Tissue engineering of bone could combine the advantages of autologous bone grafts with a minimal requirement for second interventions.

Three different approaches are currently available for treating bone defects with the aid of tissue engineering: (1) matrix-based therapy, (2) factor-based therapy, and (3) cell-based therapy. All three treatment strategies can be used either alone or in combination for reconstruction or regeneration of bone.

*Replacement of bone matrix—osteoconduction*

Matrix-based treatment approaches require implants that replace the missing bone structure with the highest possible precision. After implantation, bone precursor cells and osteoblasts should migrate into the implant material. This process is designated as osteoconduction (30). The development of biocompatible ceramic materials like hydroxyapatite and tricalcium phosphate enabled the clinical application of osteoconduction for regeneration of bone (21,72). The porous structure of the biomaterials used facilitates not only the ingrowth of cells but also their 3D orientation in the implant. The decisive disadvantage of a procedure aimed only at replacing the bone matrix is the lack of biological activity, for example, osteoinduction. This is the main objective of factor-based treatment strategies.
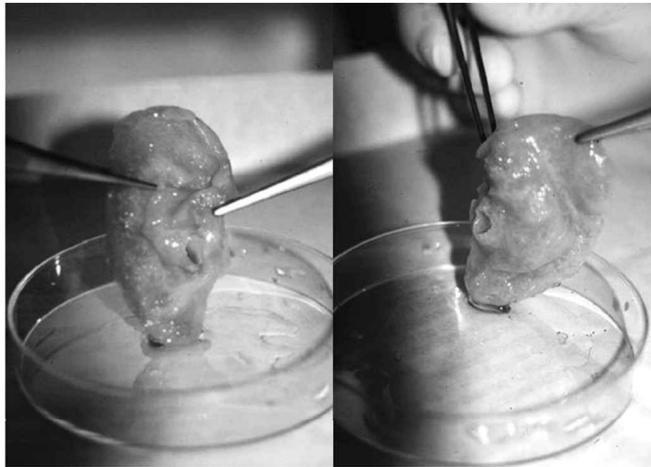
**FIG. 3.** Tissue engineered auricular construct in the front and back view. Fibrin gels were combined with resorbable polymer scaffolds for fabrication (64).

*Osteoinductive proteins—bone morphogenetic proteins*

In the sixties, Urist was able to demonstrate that reproducible intramuscular (heterotopic) osteoinduction by bone implants is only possible after demineralizing the bone (173). Partial or complete decalcification of the bone tissue enables osteoinductively effective proteins to diffuse from the bone matrix and induce cell differentiation in the surrounding tissue (125,174).

Wozney *et al.* first isolated, characterized, and cloned some of the bone morphogenetic proteins (BMPs) from bone at the end of the eighties (191). Of the more than 15 BMPs identified thus far, only six (BMP-2 to -7) have osteogenic properties. BMP-2 and BMP-7 are considered to be the strongest osteoinductive proteins. Based on their amino acid sequence, the BMPs are largely assigned to the protein family of the growth factor TGF-$\beta$ (34,131). BMPs induce chemotaxis of undifferentiated perivascular mesenchymal cells in soft tissue and undifferentiated stem cells in bone marrow by binding of the BMP mole-
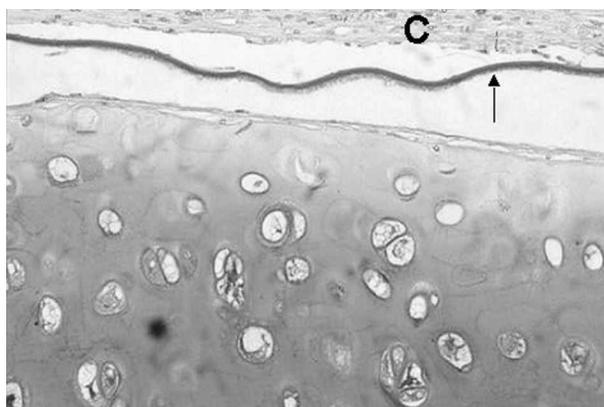
cule to specific serine/threonine kinase receptor complexes at their membrane surface (143). The increase of this receptor binding leads to proliferation of the target cells with subsequent differentiation into precursor chondrocytes and osteocytes. Within a few days enchondral ossification then leads to the development of bone tissue that can transform functionally and contains hematopoietic bone marrow. BMPs offer manifold modes of application for bone replacement. Bone formation can be induced orthotopically as well as heterotopically (in muscle tissue, for example). Bone can thus be formed at a distance from the defect through intramuscular BMP injection and then later transplanted into the bone defect freely or with a vessel (89,166).

An important prerequisite for clinical application of osteoinductive proteins is the development of suitable release systems and scaffold materials. Different biomechanical demands must be considered in connection with the implant bed. Investigations with *Escherichia coli*-expressed BMP-2 have shown that bone induction can be achieved with nearly all commercially available bone replacement matrices (94,146). BMPs have now been approved for orthopedic indications in Australia. In Europe, they will probably be applied for ENT interventions, maxillofacial surgery, and dental implantology (Figs. 5 and 6) within the next 2–3 years at the earliest (167,168).

*Osteoblasts and mesenchymal stem cells*

The first cell-based concepts for bone replacement were realized with unfractionated fresh autologous or donor bone marrow (81,127,132,184,190). Bone marrow contains osteogenic precursor cells that have the potential for inducing bone regeneration (10). In the clinical application, bone marrow is obtained from the iliac crest and implanted directly into the bone defect. For tissue engineering, precursor cells from bone marrow as well as differentiated osteoblasts can be combined with biomaterials such as demineralized bone matrix.

Osteoblasts are either take from bone biopsies or obtained through predifferentiation of precursor cells or periosteal cells—by addition of dexamethasone and ascorbic acid, for ex-
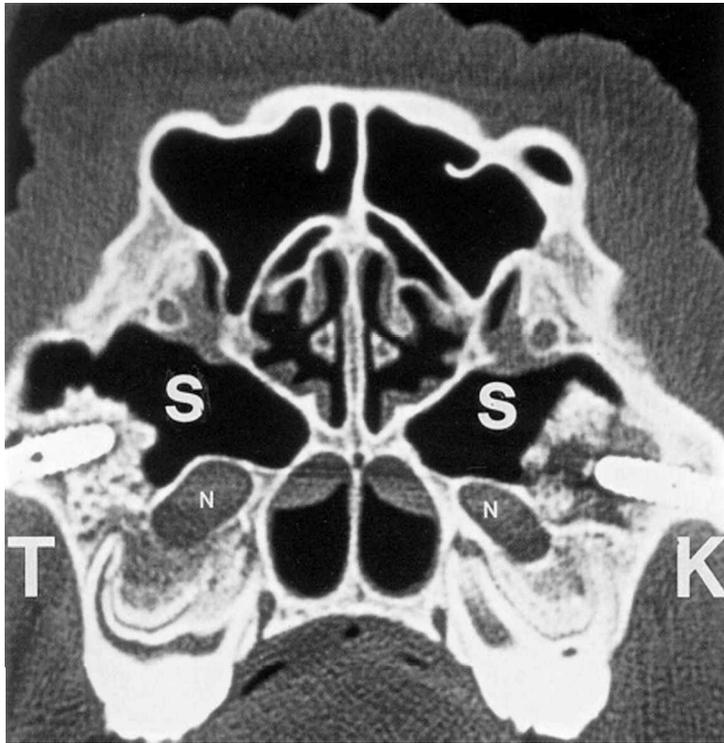


**FIG. 4.** Native cartilage with polyelectrolyte membrane encapsulation ( $\rightarrow$ ) and surrounding connective tissue (**C**) after 8 weeks *in vivo*.

**FIG. 5.** Postoperative computed tomography after experimental sinus floor augmentation and placement of dental titanium implants in miniature pigs without autologous bone transplants. Miniature pigs have a maxillary sinus (S) similar to humans. On the test side (T), recombinant osteogenic protein-1 (rhOP-1 corresponds to BMP-7) and bovine spongy bone mineral (BioOss®) were inserted submucosally into the sinus floor. The control side (K) was managed with BioOss® and a placebo solution. Augmentation of the maxillary sinus floor can be seen on both sides (167) (with permission of Dr. H. Terheyden, Department of Maxillofacial Surgery of the University of Kiel, Germany).

ample (18,108). As in the cultivation of cartilage, osteoblasts are seeded onto biodegradable polymer constructs *in vitro*. In the animal model, a full-layer skull defect could be managed by a polyglycolic acid scaffold seeded with predifferentiated osteoblasts. Marked bone regeneration was evident *in vivo* 12 weeks after implantation (15). For biomechanical reasons, slowly degradable or nondegradable scaffold materials were also tested for tissue engineering of bone (155). PET was one of the scaffold materials examined for human osteoblasts in the light of defined geometrical properties (tensile strength, mesh size). The differentiation of osteoblasts could be influenced by the appropriate surface modification, for example, hydroxyapatite coating (141).

Various study groups have described techniques for isolating human stem cells from bone marrow or periosteum in the last 10 years (18,69,122,135). These cells possess a decidedly high proliferation potential and can differentiate in the direction of highly diverse tissues such as bone, cartilage, tendons, muscles, or fat. The isolation of mesenchymal stem cells (MSC) is carried out with the aid of density gradient centrifugation and cell culturing techniques to separate the adherent MSC from the nonadherent cells. Human MSC show a characteristic pattern of different cell surface markers like growth factors, cytokine receptors, integrins, and other adhesion molecules (19,135). It is now possible to grow more than 30 populations *in vitro* from

the bone marrow aspirate of an adult MSC and to thus achieve cell counts exceeding one billion. During this expansion process, the cells remain phenotypically stable without losing their osteogenic or chondrogenic potential (18,135). In experimental studies, femoral defects in rats and rabbits were repaired using hydroxyapatite scaffolds seeded with MSC. Compared to the control group without MSC, the group with MSC-seeded implants showed bone regeneration and better biomechanical properties. The cell-free controls had good vascularization but no appreciable osteoneogenesis (20,85).

The future clinical application of bone generated *in vitro* can basically be achieved not only with osteogenic precursor cells and MSC but also with differentiated osteoblasts. It has not yet been clarified which cell type has the greatest proliferation and differentiation potential *in vitro*, and may thus be regarded as the ideal cell source for tissue engineering of bone.

## RESPIRATORY EPITHELIUM

The use of respiratory epithelium generated *in vitro* is mainly indicated in reconstructive surgery of the trachea and larynx. The management of tracheal defects is still inadequate, if their length exceeds the critical threshold for an end-to-end anastomosis as for example after resection of a tracheal stenosis. De-
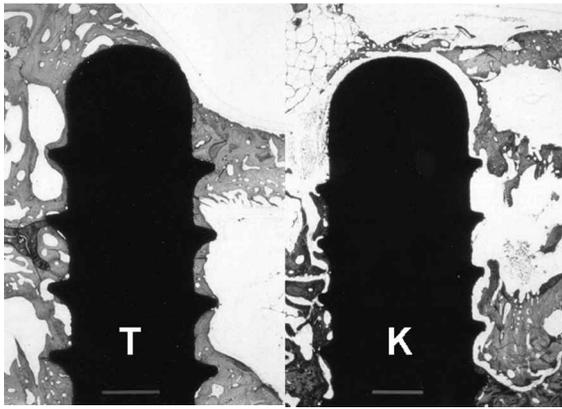
**FIG. 6.** Compared to the control side, RhOP-1 improves the osseous integration of the implants (calcified hard surface, toluidine blue—with permission of Dr. H. Terheyden, Department of Maxillofacial Surgery of the University of Kiel, Germany).

nasal septum (40,47,165), or even laryngeal transplantation (163). For these indications, future tissue engineering could provide specific replacement tissue-like composite grafts from cartilage and respiratory epithelium as an epiglottis or other laryngeal structures. Such composite grafts could also be used to close septum perforations.

## Cell and tissue culture of human respiratory epithelium

As a cell suspension, cultivated epithelium of the nasal mucosa forms a monolayer without a basal membrane. In the further course, the epithelial cells become cubic and lose their cilia (83,192,193). This phenomenon was confirmed in both *in vitro* and animal experimental studies. After cell seeding, that is, after placing a suspension of single epithelial cells on scaffold material, for example, in the lumen of a tracheal prosthesis, they could be coated with cubic to highly prismatic epithelium. Epithelial cells with normal ciliary activity were not detected (87,129). Too low a cell density of the seeded nasal mucosa cells (84,142) and insufficient supplementation of the culture medium with growth-promoting factors like vitamin A (35) have been discussed as possible factors responsible for the loss of the cilia in the monolayer cultures. Moreover, the epithelial cells are detached from the basal membrane by enzymatic separation, but since this has a regulating effect on the differentiation of epithelial cells (14,68), the loss of this cell–matrix interaction can be seen as another decisive factor for the dedifferentiation of the nasal mucosa cells in the monolayer culture. The use of tissue cultures may be a solution to this problem (Fig. 7). Small pieces of tissue about $2 \times 2$–$4 \times 4$ mm in size are cultivated here instead of a suspension from single cells. Due to the fact that connective tissue always remains attached to the epithelial explant with this approach, fibroblasts initially sprout from the piece of tissue. Ciliated cells gradually proliferated in this fibroblast layer (23). Although this system has the advantage of better *in vitro* differentiation of respiratory epithelium in the tissue culture, it fails to provide adequate cell proliferation for tissue engineering. Combining certain elements of both culturing procedures, such as *in vitro* reaggregation of single nasal mucosa cells to cell clusters (83), should be examined as another alternative for tissue engineering. Additional

spite extensive studies, it has not yet been possible to develop tracheal prostheses for long-term clinical application (12). The risks of using alloplastic materials in the trachea include rapid obstruction of the prosthesis lumen by ingrowing connective tissue with the formation of a new stenosis, defective tissue integration in the anastomotic region, and long-term rejection (42,77). Moreover, the problem of coating the prosthesis lumen with a functioning respiratory epithelium has not yet been solved. Together with the sol-gel layer, the ciliary beat is the decisive factor for mucociliary clearance in the respiratory tract (6), which should not be impaired even after reconstructive surgery of the trachea (70). Bioartificial respiratory epithelium could be used for functionalizing tracheal prostheses as well as direct epithelial coverage for scar prophylaxis after laser surgery of shorter stenoses (46).

The restoration of individual laryngeal functions after partial resection or laryngectomy usually requires extensive reconstructive measures like the use of free microvascular transplants (62,63), autologous composite grafts from the trachea or
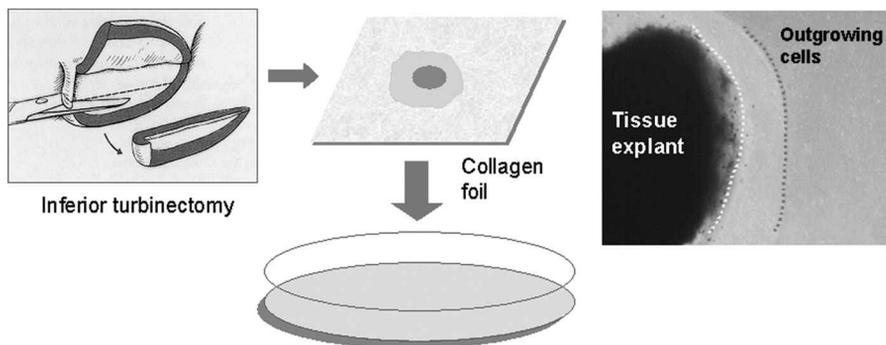


**FIG. 7.** Tissue culture of human nasal mucosa. Mucosa explants isolated from conchotomy preparations and placed on a scaffold material like collagen film. Fibroblasts initially grow from the explant edge (dotted light line) to the collagen film edge. Epithelial cells (dotted dark line) with detectable ciliary beat grow on this feeder layer (23).

optimization can be achieved by less aggressive enzymatic cell separation or by using the most physiological culturing procedures possible like air–liquid interphase culture (80,151). In the future, a pluripotent cell population may even be created by isolation and proliferation of stem cells from the deeper airways (49).

*Scaffold materials for respiratory epithelium*

As in the cultivation of skin grafts (104), collagen matrices are used as scaffold materials for tissue engineering of respiratory epithelium. They partially fulfill the function of mesenchymal components *in vitro.* Studies on embryonal epithelial tissue have repeatedly shown that direct epithelial–mesenchymal contact is required for the development of a basal membrane (5,59). A collagen scaffold used in the cultivation of human nasal mucosa explants primarily supports the adhesion and proliferation of fibroblasts. The resultant autologous fibroblast layer serves as a feeder layer for the epithelial cells. This interaction between connective tissue components and respiratory epithelium is an important prerequisite for the formation of tissue-specific features like ciliary beat (67). In our studies, epithelial cells seeded on collagen film had an active ciliary beat for up to 8 weeks *in vitro* (23).

Collagen scaffold structures do not have much mechanical strength, if they are not reinforced by other biomaterials. However, the tissue construct must be sufficiently stable, if bioartificial respiratory epithelium is to be used, for example, in future endoscopic transplantations. This could be achieved with slowly degradable or nondegradable scaffold materials. In 1992, Chopra *et al.* examined *in vitro* the possibility of attaching Dacron®-coated polyurethane with tracheal mucosa taken at autopsies. Epithelial cells grew on the prosthesis material after 5 to 7 days, but ciliated cells were not detected among the seeded cells (35). Although clinically successful in vascular prostheses for over 30 years, PET has not proven suitable as a material for tracheal prostheses particularly due to frequent restenosis and insufficient epithelial sprouting (61,77). Scaffold material porosity is an important impact parameter for preserving the tissue-specific features of the respiratory epithelium. Human nasal mucosa explants on PET nets with a mesh size of 50 to 300 $\mu$m had the highest ciliated cell density on nets with a 150-$\mu$m mesh size. The growth behavior of fibroblasts sprouting from the tissue explant was also markedly dependent on the mesh size of the textile scaffold. Fibroblasts grew more rapidly and more densely on nets with narrower mesh sizes (50–100 $\mu$m). In comparison, reduced, "controlled" seeding of fibroblasts on nets with 150-$\mu$m mesh size seems to have a positive effect on the differentiation of epithelial cells (24,141).

Complete *in vitro* generation of human tracheal (93) segments may not be possible, since the mechanical properties of connective and supporting tissues produced *in vitro* have not yet been identified. However, it may soon be possible to repair circumscribed epithelial defects like those after laser removal of short tracheal stenoses or velum formations.

## ORAL MUCOSA

Radial forearm flaps and jejunum grafts are among the most common free transplants used for reconstruction of the upper aerodigestive tract after tumor operations. Despite their widespread clinical use, skin and jejunum mucosa are not ideal for mucosa replacement in the aerodigestive tract. Both types of tissue easily dry out, and may thus lead to more difficult food passage during swallowing. Moreover, the possible hairiness of a skin transplant is very disturbing in the oral cavity. A solution for these problems may be the prelamination of forearm flaps with keratinocytes from the oral mucosa (101). In tissue culture, oral keratinocytes growing from mucosa biopsies are seeded on collagen film after 14 days. After another 2 days, this construct is implanted in a subcutaneous pouch at the donor site of the forearm flap. The oral keratinocytes are thus in direct contact with the forearm fascia, and the film above it is next to the skin. Following a healing period, only the kerotinocyte-containing fascia segment is lifted with the vessel pedicle. The donor site can then be primarily closed using the previously tunneled forearm skin with the advantage that complete skin removal, for example, from the groin is no longer necessary. Only a few patients have thus far been managed with such prelaminated fascia flaps. A normal mucosa epithelium was detected both clinically and histologically. No data is presently available on whether this bioartificial mucosa will survive adjuvant radiotherapy.

In oral and maxillofacial surgery, mucosa transplants fabricated *in vitro* have been used since the beginning of the 1990s to manage intraoral mucosa defects (100,172).

## SALIVARY GLANDS

All diseases accompanied by permanently reduced salivation are possible treatment targets for tissue engineering. Radiogenic xerostomia after radiotherapy of malignant head and neck tumors is of particular importance here due to the high number of affected patients. The number of new diseases is estimated to be over 500,000 cases worldwide (181). Causal treatment options for radiation-induced salivary gland damage are not yet available; thus, various study groups are currently investigating, whether cell therapy concepts can be developed with tissue engineering methods (7,25).

The basic requirement for their therapeutic application is the *in vitro* preservation and increase of functional, that is, saliva-producing, tissue. The first trials with static tissue cultures of the human submandibular gland showed a significant increase of autolytic processes after 48 h *in vitro* (78,79). The culture duration was extended to 2–3 weeks with the preservation of differentiated submandibular tissue by using fibronectin, laminin, and collagen type I and IV as matrix components and by adding various growth factors like EGF and FGF (97,158). In tissue cultures of human submandibular and parotid tissue, Dardick *et al.* determined that basically all salivary gland cell types (acinic cells, ductal epithelia, and myoepithelia) can be cultured within 28 days and proliferated by adding the $\beta$-adrenergic drug isoprotenerol (IPR) (37). Additional effects of IPR stimulation were a high squamous metaplasia rate of cultivated salivary gland cells [37] and stimulation of amylase secretion *in vitro* (36). However, it is doubtful whether sufficient amounts of functional salivary gland cells can be achieved for seeding on scaffold materials due to the great difficulty of growing human salivary gland tissue *in vitro* (152).

Tissue engineering concepts for salivary glands were presented independently by two different study groups for the first time in 1998. Microcarriers were seeded with individual enzymatic human parotid cells with the intention of fabricating autologous salivary gland organoids for minimally invasive application, for example, for injection into the oral mucosa of irradiated patients (22). The parotid cells retained their tissue-specific differentiation properties for up to 3 weeks *in vitro* on the two microcarriers used here. In all adherent cells, cytokeratin was detected as a sign of epithelial differentiation. Differentiation was assumed to be preserved in this cell population because of the high concentrations of tissue polypeptide antigens in medium supernatant, a specific marker of ductal epithelia in salivary glands. However, this concept for fabricating salivary gland organoids cannot be used for therapeutic purposes (26) due to the amylase decrease in the medium indicating a continuous reduction in the secretory functions of acinic cells *in vitro*. Comparable results were achieved after implantation of a collagen sponge seeded with salivary gland cells in an animal model. Amylase production was detected up to 3 weeks after implantation (86). These results show that it is currently possible to maintain vital and functional salivary gland tissue both *in vitro* and *in vivo* for a relatively short time. Thus, a clinical application still appears to be unrealistic at the moment. The use of an allogeneic immortalized submandibular cell line (HSG) has been suggested as an alternative to autologous salivary gland cells (7). Different polymers (PLA, PGA) were seeded with HSG cells *in vitro* (1). A tube closed at one end made of a porous slowly degradable biomaterial is being considered as a scaffold for clinical use. However, the problem is the almost unlimited growth potential of HSG cells developed from a tumor cell line (159). Clinical application of these cells is contraindicated due to their incalculable oncogenic potential. Considering the problems described here, causal cell-based therapy of radiation-induced xerostomia still remains a vision of the future.

## CONCLUSIONS AND PERSPECTIVES

Tissue engineering opens up new ways to generate vital and functional transplants. Various basic problems must still be solved before clinically applying *in vitro* fabricated tissue. Only a fraction of all somatic organ-specific cell types can be grown in sufficient amounts *in vitro* (Table 1). Availability, isolation, and proliferation of human stem cells thus play a decisive role in tissue engineering (99). Whether pluripotent adult precursor cells or embryonal stem cells will be available in the future not only depends on advances in research but also on political decisions. The combination of genetic and tissue engineering methods, for example, in the generation of immortalized cells, may be another option for developing cell-based therapeutic procedures (120,185).

The inadequate *in vitro* oxygen and nutrition supply is another limiting factor for the fabrication of complex tissues or organ systems. Tissue survival is doubtful after implantation, if its supply is not ensured by a capillary network.

TABLE 1.  CULTIVABILITY AND *IN VITRO* PROLIFERATION POTENTIAL OF DIFFERENT PRIMARY HUMAN CELLS[a]

| Organ/tissue | Characteristic cell types | Cultivability proliferation potential in vitro |
|---|---|---|
| Peripheral nerves CNS | Neurons, astrocytes | Cultivable for several weeks, limited growth |
| Heart | Cardiomyocytes | Cultivable for several weeks, appreciable proliferation only in fetal cardiomyocytes |
| Vessels | Vascular fibroblasts, endothelial cells, smooth muscle cells | Cultivable for weeks to months, relatively high proliferation capacity |
| Nose, trachea | Respiratory epithelial cells | Cultivable in differentiated form for up to 8 weeks as a tissue culture, limited growth |
| Lungs | Pneumocytes | Cultivable for at least 1 week, *in vitro* proliferation of adult pneumocytes not yet possible |
| Liver | Hepatocytes | Cultivable for several weeks, appreciable proliferation potential only in fetal hepatocytes |
| Pancreas | Islet cells | Difficult to isolate larger amounts of cells, sufficient growth is not yet possible *in vitro* |
| Salivary glands | Duct epithelium, acinar cells, myoepithelia | Cultivable for up to 6 weeks, detectable proliferation only in duct epithelium |
| Intestine | Enterocytes, endocrine cells | High proliferation potential due to organ-specific precursor cells in isolated intestinal cells |
| Kidney | Epithelial cells of renal tubules | Limited cultivation and proliferation potential |
| | Mesangial cells | Cultivable for weeks to months, relatively high proliferation capacity |
| Bladder Ureter | Urothelial cells | Readily cultivable, good proliferation on "feeder cells" |
| Skin Hair | Skin fibroblast, epithelial cells of the epidermis | Cultivable for several months, high proliferation capacity |
| Cartilage | Chondrocytes | Cultivable for weeks to months, relatively high proliferation capacity, rapid dedifferentiation *in vitro* |
| Bone | Osteoblasts | Good cultivability, high proliferation capacity |

[a]Modified from (112).

In the meantime, tissue engineering has also gained considerable economic importance. As in other biotechnological branches, an increasing number of new companies will be established in the coming years. According to a survey by the journal *Business Week* (issue from July 27, 1998, p. 61), the market potential of tissue engineering products in the United States is estimated to be 80 billion dollars (179). In 1998, the capital assets of American tissue engineering companies exceeded 3.5 billion dollars with an annual growth rate of 22.5% (107).

Tissue engineering companies or institutes will settle at appropriately certified hospitals to grow replacement tissue under GMP conditions close to clinical partners. Only close cooperation between disciplines in science, medicine, and industry will enable the clinical application of results obtained from tissue engineering research.

## REFERENCES

1. Aframian DJ, Cukierman E, Nikolovski J, Mooney DJ, Yamada KM, Baum BJ. (2000). The growth and morphological behaviour of salivary gland epithelial cells on matrix protein-coated biodegradable substrata. *Tissue Eng* 3:209–216.
2. Aigner J, Tegeler J, Hutzler P, Campoccia D, Pavesio A, Hammer C, Kastenbauer E, Naumann A. (1998). Cartilage tissue engineering with novel nonwoven structured biomaterial based on hyaluronic acid benzyl ester. *J Biomed Mater Res* 42:172–181.
3. Armstrong CG, Mow VC. (1982). Variations in the intrinsic mechanical properties of human articular cartilage with age, degeneration and water content. *J Bone Joint Surg Am* 64:88–94.
4. Aulthouse AL, Beck M, Griffey, Sanford J, Arden K, Machado MA, Horton WA (1989). Expression of the human chondrozyte phenotype *in vitro*. *In Vitro Cell Dev Biol* 25:659–668.
5. Baeza-Squiban A, Romet S, Moreau A, Marano F. (1991). Progress in outgrowth culture from rabbit tracheal explants: Balance between proliferation and maintenance of differentiated state in epithelial cells. *In Vitro Cell Dev Biol* 27A:453–460.
6. Bals R. (1997). Zelltypen der Atemwegsepithelien: Morphologie, Molekularbiologie und klinische Bedeutung. *Pneumologie* 51:142–149.
7. Baum BJ, Wang S, Cukierman E, Delporte C, Kagami H, Marmary Y, Fox PC, Mooney DJ, Yamada KM. (1999). Re-engineering the functions of a terminally differentiated epithelial cell in vivo. *Ann NY Acad Sci* 874:294–300.
8. Bell E, Ehrlich P, Buttle DJ, Nakatsuji T. (1981). Living tissue formed *in vitro* and accepted as skin-equivalent of full thickness. *Science* 221:1052–1054.
9. Benja PD, Schaffer JD. (1978). Dedifferentiated chondrocytes re-express the differentiated collagen phenotype when cultured in agarose gels. *Cell* 30:215–224.
10. Beresford JN. (1989). Osteogenic stem cells and the stromal system of bone and marrow. *Clin Orthop* 240:270–280.
11. Berghaus A. (1992). Alloplastische Implantate in der Kopf-Halschirurgie. *Eur Arch Otorhinolaryngol Suppl* 1:53–95.
12. Berghaus A. (1992). Eine neue Trachealprothese. *Otorhinolaryngol Nova* 2:232–237.
13. Bobic V. (2000). Current status of the articular cartilage repair. *E-Biomed* 1:37–41.
14. Bohnert A, Hornung J, Mackenzie IC, Fusenig NE. (1986). Epithelial-mesenchymal interactions control basement membrane production and differentiation in cultured and transplanted mouse keratinocytes. *Cell Tissue Res* 244:413–429.
15. Breitbart AS, Grande DA, Kessler R, Ryaby JT, Fitzsimmons RS, Grant RT. (1998). Tissue engineering bone repair of calvarial defects using cultured periosteal cells. *Plast Reconstr Surg* 101:567–574.
16. Brittberg M, Lindahl A, Nilson A, Ohlsson C, Isakson O, Peterson L. (1994). Treatment of deep cartilage effects in the knee with autologous chondrocyte transplantation. *N Eng J Med* 331:889–895.
17. Bruckner P, Hörler I, Mendler M, Houze Y, Winterhalter KH, Eich-Bender SG, Spycher MA. (1989). Induction and prevention of chondrocyte hypertrophy in culture. *J Cell Biol* 109:2537–2545.
18. Bruder SP, Jaiswal N, Haynesworth SE. (1997). Growth kinetics, self-renewal and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 64:278–294.
19. Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S. (1998). Mesenchymal stem cells in osteobiology and applied bone regeneration. *Clin Orthop* 355(Suppl):S247–S256.
20. Bruder SP, Kraus KH, Goldberg VM, Kadiyala S. (1988). The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg* 80A:985–996.
21. Buchholz RW, Carlton A, Holmes RE. (1987). Hydoxyapatite and tricalcium phosphate bone graft substitutes. *Orthop Clin North Am* 18:323–334.
22. Bücheler M, Bootz F. (2000) Biotechnologisch hergestellte Speicheldrüsen-Organoide. Patent, Publication-No.:DE19838495 Date of publication: 02. 03. 2000.
23. Bücheler M, Scheffler B, von Foerster U, Bruinink A, Bootz F, Wintermantel E. (2000). Wachstum humanen respiratorischen Epithels auf Kollagenfolie. *Laryngo Rhino Otol* 79:160–164.
24. Bücheler M, Raeber G, Mayer J, Scheffler B, Wintermantel E, Bootz F. (2000). Scaffold design for respiratory epithelium transplants. In: *4th European Congress of Oto-Rhino-Laryngology Head and Neck Surgery*. Jahnke K, Fischer M, eds. Berlin: Bologna pp. 1355–1359.
25. Bücheler M. (2001). Entwicklung biotechnologischer Therapieverfahren für die kausale Behandlung der radiogenen Xerostomie. *Laryngo Rhino Otol* 80:637–638.
26. Bücheler M, Wirz C, Schütz A, Bootz F. (2002). Tissue engineering of human salivary gland organoids. *Acta Otolaryngol* 122:541–545.
27. Bujía J, Pitzke P, Wilmes E, Hammer C. (1992). Culture and cryopreservation of chondrocytes from human cartilage: Relevance for cartilage autografting in otolaryngology. *ORL J Otorhinolaryngol Relat Spec* 54:80–84.
28. Bujía J, Alsalameh A, Sittinger M, Hammer C, Wilmes E, Burmester G. (1994). Humoral immune response against minor collagens type IX and XI in patients with cartilage graft resorbtion after reconstructive surgery. *Ann Rheum Dis* 53:229–234.
29. Bujía J, Rotter N, Minuth W, Burmester G, Hammer C, Sittinger M. (1995). Züchtung menschlichen Knorpelgewebes in einer dreidimensionalen Perfusionskulturkammer: Charkterisierung der Kollagensynthese. *Laryngo Rhino Otol* 74:559–563.
30. Burchardt H. (1983). The biology of bone graft repair. *Clin Orthop* 174:28–42.
31. Burke JF, Yannas IV, Quimby WC, Bondoc CC, Jung WK. (1981). Sucessful use of physiologically acceptable artificial skin in the treatment of extensive burn injury. *Ann Surg* 194:413–448.
32. Busse B, Smith MD, Gerlach JC. (1999). Treatment of acute liver failure: Hybrid liver support. A critical overview. *Langenbecks Arch Surg* 384:588–599.
33. Castagnola P, Dozin B, Moro G, Cancedda R. (1988). Changes

in the expression of collagen genes show two stages in chondrocyte differentiation *in vitro*. *J Cell Biol* 106:461–467.

34. Celeste JA, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM. (1990). Identification of transforming growth factor $\beta$ family members present in bone-inductive protein puriefied from bovine bone. *Proc Natl Acad Sci USA* 87:9843–9847.

35. Chopra DP, Kern RC, Mathieu PA, Jacobs JR. (1992). Sucessful *in vitro* growth of human respiratory epithelium on a tracheal prosthesis. *Laryngoscope* 102:528–531.

36. Chopra DP, Xue-Hu IC. (1993). Secretion of $\alpha$-amylase in human parotid gland epithelial cell culture. *J Cell Physiol* 155:223–233.

37. Dardick I, Dardick AM, MacKay AJ, Pastolero GC, Gullane PJ, Burford-Mason AP. (1993). Pathobiology of salivary glands. IV. Histogenetic concepts and cycling cells in human parotid and submandibular glands cultured in floating collagen gels. *Oral Surg Oral Med Oral Pathol* 76:307–318.

38. Dautzenberg H, Arnold G, Tiersch B, Lukanoff B, Eckert U. (1996). Polyelectrolyte complex formation at the interface of solutions. *Prog Coll Polym Sci* 101:149–156.

39. Dautzenberg H, Stange J, Mintzer S, Lukanoff B. (1996). Encapsulation by polyelectrolyte complex formation—A way to make hepatocyte cultures safe, efficient and available. In *Immobilized Cells, Basics and Application in Process in Biotechnology*. H. Dautzenberg, ed. Elsevier Science: New York, pp. 181–188.

40. Delaere PR, Vander Poorten V, Vanclooster C, Goeleven A, Hermans R. (2000). Results of larynx preservation surgery for advanced laryngeal cancer through tracheal autotransplantation. *Arch Otolaryngol Head Neck Surg* 126:1207–1215.

41. De Palma AF, McKeever CD, Subin DK. (1966). Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin Orthop* 48:229–242.

42. Donald PJ. (1998). Trachealchirurgie. Chronische Stenosen. In: Kopf-und Halschirurgie, *Band 3: Hals*. Naumann HH, Helms J, Herberhold C, Jahrdoerfer RA, Kastenbauer ER, Panje WR, Tardy ME Jr., eds. Stuttgart: Thieme, pp. 258–269.

43. 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.

44. Doyle A, Griffith JB, Newell DG. (1993). Cell and tissue culture. In: *Laboratory Procedures*. Chichester: John Wiley & Sons.

45. Duda GN, Haisch A, Endres M, Gebert C, Schröder 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.

46. Duff BE, Wenig BL, Applebaum EL, Yeates DB, Wenig BM, Holinger LD. (1994). Tracheal reconstruction using an epithelial allograft. *Laryngoscope* 104:409–414.

47. Duncavage JA, Ossoff RH, Toohill RJ. (1989). Laryngotracheal reconstruction with composite nasal septal cartilage grafts. *Ann Otol Rhinol Laryngol* 98:581–585.

48. Elima K, Vuorio E (1989). Expression of mRNAs for collagens and other matrix components in dedifferentiating and redifferentiating human chondrocytes in culture. *FEBS Lett* 258:195–198.

49. Emura M. (1997). Stem cells of the respiratory epithelium and their *in vitro* cultivation. *In Vitro Cell Dev Biol* 33:3–14.

50. Eufinger H, Wehmöller M. (1998). Individual prefabricated titanium implants in reconstructive craniofacial surgery:Clinical an technical aspects of the first 22 cases. *Plast Reconstr Surg* 102:300–308.

51. Fischer-Fröhlich CL. (1997). Die Situation der Organtransplantation in der Bundesrepublik Deutschland und im europäischen Ausland aus medizinischer Sicht—Eine Bestandsaufnahme. In: *Landeszentrale für politische Bildung (Hrsg) Organentnahme und Transplantation*. Stuttgart: Bad Urach.

52. Freed LE, Vunjak-Novakovic G, Langer R. (1993). Cultivation of cell–polymer cartilage implants in bioreactors. *J Cell Biochem* 51:257–264.

53. Freed LE, Vunjak-Novakovic G. (1995). Cultivation of cell–polymer constructs in simulated microgravity. *Biotechnol Bioeng* 46:306–313.

54. Freed LE, Vunjak-Novakovic G. (1997). Microgravity tissue engineering. *In Vitro Cell Dev Biol Anim* 33:381–385.

55. Freed LE, Langer R, Martin I, Pellis NR, Vunjak-Novakovic G. (1997). Tissue engineering of cartilage in space. *Proc Natl Acad Sci USA* 94:13885–13890.

56. Fujimoto E, Ochi M, Kato Y, Mochizuki Y, Sumen Y, Ikuta Y. (1999). Beneficial effect of basic fibroblast growth factor on the repair of full-thickness defects in rabbit articular cartilage. *Arch Orthop Trauma Surg* 199:139–145.

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

58. Grande DA. (1999). Cartilage tissue engineering:Current limitations and solutions. *Clin Orthop* 367S:176–185.

59. Grobstein C. (1967). Mechanisms of organogenetic tissue interaction. *Natl Cancer Inst Monogr* 26:279–299.

60. Gruber R, Sittinger M, Bujía J. (1996). Untersuchung 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.

61. Habal DLM, Pizzoferrato A, Vespucci A. (1985). Prosthetic replacement of large defects of the cervical trachea in dogs. *Biomaterials* 6:17–22.

62. Hagen R. (1990). Laryngoplasty with a radialis pedicle flap from the forearm. A surgical procedure for voice rehabilitation after total laryngectomy. *Am J Otolaryngol* 11:85–89.

63. Hagen R, Schwab B, Marten S. (1995). Nasotracheal airway-oropharyngeal alimentary canal:A microvascular technique for reconstruction of the upper airway after total laryngectomy. *Ann Otol Laryngol* 104:317–322.

64. Haisch A, Schultz O, Perka C, Jahnke V, Burmester GR, Sittinger M. (1996). Tissue Engineering humanen Knorpelgewebes für die rekonstruktive Chirurgie unter Verwendung biokompatibler resorbierbarer Fibringel- und Polymervliesstrukturen. *HNO* 44:624–629.

65. Haisch A, Gröger A, Radke C, Ebmeyer J, Sudhoff H, Grasnick G, Jahnke V, Burmester GR, Sittinger M. (2000). Resorptionsprotektion autogener Knorpeltransplantate durch Polyelektrolytkomplexmembranverkapselung. *HNO* 48:119–124.

66. Haisch A. Personal communication, 2001.

67. Hanamure Y, Deguchi K, Ohyama M. (1994). Ciliogenesis and mucus synthesis in cultured human respiratory cells. *Ann Otol Rhinol Laryngol* 103:889–895.

68. Hay ED. (1978). Role of basement membranes in development and differentiation. In: Kefalides NA, ed. *Biology and Chemistry of Basement Membranes*. New York Academic Press, pp. 119–136.

69. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. (1992). Characterisation of cells with osteogenic potential from human marrow. *Bone* 13:81–88.

70. Herberhold C, Stein M, Bierhoff E, Kost S. (1999). Trachealrekonstruktion mit konserviertem trachealem Homograft—Neue Aspekte. *Laryngo Rhino Otol* 78:54–56.

71. Hill NM, Horne JG, Devane PA. (1999). Donor site morbidity in the iliac crest bone graft. *Aust N Z J Surg* 69:726–728.

72. Hollinger JO, Brekke J, Gruskin E, Lee D. (1996). Role of bone substitutes. *Clin Orthop* 324:55–65.

73. Horton WE Jr, Higginbotham JD, Chandrasekhar S. (1989). Transforming growth factor-beta and fibroblast growth factor act synergistically to inhibit collagen II synthesis through a mechansim involving regulator DNA sequences. *J Cell Physiol* 141:8–15.

74. Horwitz AF. (1997). Integrins and health. *Sci Am* 276:68–75.

75. Isabelle ME, Githens S, Moses RL, Bartell CK. (1994). Culture of rat renal medullary tissue in media made hyperosmotic with NaCl and urea. *J Exp Zool* 269:308–318.

76. Ishizaki Y, Burne JF, Raff MC. (1994). Autocrine signals enable chondrocytes to survive in culture. *J Cell Biol* 126:1069–1077.

77. Jacobs JR. (1988). Investigations into tracheal prosthetic reconstruction. *Laryngoscope* 98:1239–1244.

78. Jacobson N, Brennhovd I, Jonsen J. (1977). Human submandibular gland tissue in culture 1. Sulphate incorporation and tissue culture technique. *J Biol Buccale* 5:159–167.

79. Jacobson N, Brennhovd I, Jonsen J. (1977). Human submandibular gland tissue in culture 2. Nickel affinity to secretory proteins. *J Biol Buccale* 5:169–175.

80. Jackson AD, Rayner CFJ, Dewar A, Cole PJ, Wilson R. (1996). A human respiratory-tissue organ culture incorporating an air interface. *Am J Respir Crit Care Med* 153:1130–1135.

81. Jackson IT, Scheker LR, Vandervord JG, Mc Lennan JG. (1981). Bone marrow grafting in the secondary closure of alveolar-palatal defects in children. *Br J Plast Surg* 34:422–425.

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

83. Joerissen M, van den Schueren B, van den Berghe H, Cassiman JJ. (1989). The preservation and regeneration of cilia on human cells cultured *in vitro*. *Arch Otorhinolaryngol* 246:308–314.

84. Joerissen M, Bessems A. (1995). Normal ciliary beat frequency after ciliogenesis in nasal epithelial cells cultured sequentially as monolayer and in suspension. *Acta Otolaryngol* 115:66–70.

85. Kadiyala S, Jaiswal N, Bruder SP. (1997). Culture-expanded, bone marrow-derived stem cells can regenerate a critical-sized segmental bone defect. *Tissue Eng* 3:173–185.

86. Kagami H, Hata K, Horie K, Nishiguchi H, Matsuno M, Shigetomi T, Ueda M. (1998). Tissue engineering of salivary glands. *Tissue Eng* 4:472.

87. Kaschke O, Gerhardt HJ, Bˆhm K, Wenzel M, Planck H. (1995). Die Epithelisierung poröser Biomaterialien mit isolierten respiratorischen Epithelzellen in vivo. *HNO* 43:80–88.

88. Kato Y, Hiraki Y, Inoue H, Kinoshita M, Yutani Y, Suzuki F. (1983). Differential and synergistic actions of somatmedin-like growth factors, fibroblast growth factor and epidermal growth factor in rabbit costal chondrocytes. *Eur J Biochem* 129:685–690.

89. Khouri RK, Koudsi B, Reddi AH. (1991). Tissue transformation into bone in vivo. *JAMA* 266:1953–1955.

90. Klug MG, Sonpaa MH, Koh GY, Field LJ. (1996). Genetically selected cardiomyocytes from differentiating embronic stem cells form stable intracardiac graft. *J Clin Invest* 98:216–224.

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

92. 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.

93. Kojima K, Bonassar LJ, Roy AK, Cao Y, Mizuno H, Vacanti CA, Cortiella J. (2000). A composite tissue engineered trachea using sheep nasal chondrocytes and epithelial cells. *Tissue Eng* 6:681.

94. Kübler NR, Reuther JF, Faller G, Kirchner T, Ruppert R, Sebald W. (1998). Inductive properties of recombinant human BMP-2 produced in a bacterial expression system. *Int J Oral Maxillofac Surg* 27:305–309.

95. Kuettner KE, Pauli BU, Ball G, Memoli AV, Schenk RK. (1982). Synthesis of cartilage matrix by mammalian chondrocytes in vivo. 1. Isolation, culture characteristics and morphology. *J Cell Biol* 93:743–750.

96. Kulyk WM, Rodgers BJ, Greer K, Kosher RA. (1989). Promotion of embryonic chick limb cartilage differentiation by transforming growth factor-beta. *Dev Biol* 135:424–430.

97. Kurth BE, Hazen-Martin DJ, Sens MA, Sens D. (1988). Ultrastructural and immunohistochemical characterisation of submandibular duct cells in culture and modification of outgrowth differentiation by manipulation of calcium ion concentration. *In Vitro Cell Dev Biol* 24:593–600.

98. Langer R, Vacanti JP. (1993). Tissue engineering. *Science* 260:920–926.

99. Langer R. (2000). Tissue engineering. *Mol Ther* 1:12–15.

100. Lauer G, Otten JE, Specht von BU, Schilli W. (1991). Cultured gingival epithelium. A possible suitable material for pre-prosthetic surgery. *J Cranio Max Fac Surg* 19:21–26.

101. Lauer G, Schimming R, Gellrich NC, Schmelzeisen R. (2000). Prelamination of the radial forearm flap using tissue engineered mucosa. *Cells Tissues Organs* 166:132.

102. Lim F, Sun AM. (1980). Microencapsulated islets as bioartifical endocrine pancreas. *Science* 210:908–909.

103. Lindl T. (2000). *Zell- und Gewebekultur:Einführung in die Grundlagen sowie ausgewählte Methoden und Anwendungen.* Heidelberg: Spektrum, Akad. Verlag.

104. Lópes Valle CA, Germain L, Rouabhia M, Xu W, Guignard R, Goulet F, Auger FA. (1996). Grafting on nude mice of living skin equivalents produced using human collagens. *Transplantation* 62:317–323.

105. Lu L, Mikos AG. (1996). The importance of new processing techniques in tissue engineering. *Mater Res Soc Bull* 21:28–32.

106. Lu L, Garcia CA, Mikos AG. (1999). *in vitro* degradation of thin poly(DL-lactic-co-glycolic acid) films. *J Biomed Mater Res* 46:236–244.

107. Lysaght MJ, Nguy NAP, Sullivan K. (1998). An economic survey of the emerging tissue engineering industry. *Tissue Eng* 4:231–238.

108. Maniatopoulos C, Sodek J, Melcher AH. (1988). Bone formation *in vitro* by stromal cells obtained from marrow of young adult rats. *Cell Tissue Res* 25:317–330.

109. Mantesanz R, Miranda B, eds. (1995). *Preliminary Data Report Organ Donation and Transplantation*, 1995. Madrid, Spain: Aula Medica.

110. Marchisio PC, Capasso O, Nitsch I, Cancedda R, Gionti E. (1984). Cytoskeleton and adhesion patterns of cultured chick embryo chondrocytes during cell spreading and Rous sarcoma virus transformation. *Exp Cell Res* 151:332–343.

111. Mayne R, Vail M, Mayne PM, Miller EJ. (1976). Changes in the type of collagen synthesized as clones of chick chondrocytes grow and eventually lose division capacity. *Proc Natl Acad Sci USA* 73:1674–1678.

112. Mertsching H, Martin U. (2001). Zelltransplantation und Tissue Engineering:Aktuelle Entwicklungen und klinische Perspektiven. *transkript Laborwelt* 1:4–11.

113. Meyer R. (1998). Diskussion um Xenotransplantation:Tierorgane als trojanische Pferde für Erreger. *Dt Ärztbl* 95:A-1895–1896.

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

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

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

117. Minuth WW, Sittinger M, Kloth S. (1998). Tissue engineering: Generation of differentiated artifical tissues for biomedical applications. *Cell Tissue Res* 291:1–11.

118. Minuth WW, Schumacher K, Strehl R, Kloth S. (2000). Physiological and cell biological aspects of perfusion culture technique employed to generate differentiated tissues for long term biomaterial testing and tissue engineering. *J Biomater Sci Polymer Edn* 11:495–522.

119. Mow VC, Holmes MH, Lai WM. (1984). Fluid transport and mechanical properties of articular cartilage: A review. *J Biomech* 17:377–394.

120. Mulligan R. (1993). The basic science of gene therapy. *Science* 260:926–932.

121. Nagata S. (1994). Modification of the stages in total reconstruction of the auricle: Part I–IV. *Plast Reconstr Surg* 93:221–266.

122. Nakahara H, Bruder SP, Goldberg VM, Caplan AI. (1989). In vivo osteochondrogenic potential of cultured cells derived from the periosteum. *Clin Orthop Scand* 259:223–232.

123. Naumann A, Bujía J, Hammer C, Wilmes E. (1994). Autoantibodies against cartilage components:Clinical relevance for head and neck surgery. *Laryngo Rhino Otol* 73:253–257.

124. Nimni ME. (1997). Polypeptide growth factors:Targeted delivery systems. *Biomaterials* 18:1201–1225.

125. Nogami H, Urist MR. (1975). Transmembrane bone matrix gelatin-induced differentiation of bone. *Calcif Tissue Res* 19:153–163.

126. Ohara K, Nakamura K, Ohta E. (1994). Chest wall deformities and thoracic scoliosis after costal cartilage graft harvesting. *Plast Reconstr Surg* 93:1030–1036.

127. Ohgushi H, Goldberg VM, Caplan A. (1989). Repair of bone defects with marrow cells and porous ceramics. *Acta Orthop Scand* 60:334–339.

128. O'Keefe RJ, Crabb ID, Puzas JE, Rosier RN. (1994). Effects of transforming growth factor-beta 1 and fibroblast growth factor on DNA synthesis in growth plate chondrocytes are enhanced by insulin-like growth factor-1. *J Orthop Res* 12:299–310.

129. Olze H, Kaschke O, Müller WD. (1997). Untersuchungen zur Verbesserung des Designs eines allplastischen epithelisierten Tracheaersatzes. *HNO* 45:453–459.

130. Osborn KD, Trippel SB, Mankin HJ. (1989). Growth factor stimulation of adult cartilage. *J Orthop Res* 7:35–42.

131. Özkaynak E, Rueger DC, Drier EA, Corbett C, Ridge RJ, Sampath TK, Oppermann H. (1990). OP-1 cDNA encodes an osteogenic protein in the TGF-$\beta$ family. *EMBO J* 9:2085–2093.

132. Paley D, Young MC, Wiley AM, Fornasier VL, Jackson RW. (1986). Percutaneous bone marrow grafting of fractures and bony defects: An experimental study in rabbits. *Clin Orthop* 208:300–312.

133. Park SS. (2000). Reconstruction of nasal defects larger than 1,5 centimeters in diameter. *Laryngoscope* 110:1241–1250.

134. Peiseler B. (2001). Ohrmuscheln und Gelenke aus Zellkultur. *Baseler Zeitung* 16:55–57.

135. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* 284:143–147.

136. Posever J, Phillips FM, Pottenger LA. (1995). Effects of basic fibroblast growth factor, transforming growth factor-beta 1, insulin like growth factor-1, and insulin on human osteoarthitic articular cartialge explants. *J Orthop Res* 13:832–837.

137. Prewett TL, Goodwin TJ, Spaulding GF. (1993). Three-dimensional modeling of T-24 human bladder carcinoma cell line:A new simulated microgravity culture system. *J Tissue Cult Methods* 59:29–36.

138. Psillakis JM, Nocchi VLB, Zanini SA. (1979). Repair of large defects of frontal bone with free graft of outer table of parietal bones. *Plast Reconstr Surg* 64:827–830.

139. Puelacher WC, Wisser J, Vacanti CA, Ferraro NF, Jaramillo D, Vacanti JP. (1994). Temporomandibular joint disc replacement made by tissue engineered growth of cartilage. *J Oral Maxillofac Surg* 52:1172–1177.

140. 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.

141. Raeber G, Bücheler M, Scheffler B, Karamuk E, Mayer J, Bootz F, Wintermantel E. (2000):PET-fabrics as a model scaffold for tissue engineering. *Cells Tissues Organs* 166:128–129.

142. Rautiainen M, Matsune S, Yoshitsugu M, Ohyama M. (1993). Degeneration of human respiratory cell ciliary beat in monolayer cell culture. *Eur Arch Otorhinolaryngol* 250:97–100.

143. Reddi AH. (1997). Bone morphogenetic proteins: An unconventional approach to isolation of first mammalian morphogens. *Cytokine Growth Factor Rev* 8:11–20.

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

145. Rettinger G. (1992). Autogene und allogene Knorpeltransplantate in der Kopf- und Halschirurgie (ohne Mittelohr und Trachea). *Eur Arch Otorhinolaryngol Suppl* 1:127–162.

146. Reuther JF, Kübler NR. (1999). Die Wiederherstellung des Unterkiefers. *Dt Ärztbl* 96:A-1054–1061.

147. Rheinwald JG, Green H. (1975). Serial cultivation of strains of human epidermal keratinocytes: The formation of keratinizing colonies from single cells. *Cell* 6:331–343.

148. Rosen DM, Stempien SA, Thompson AY, Seyedin SM. (1988). Transforming growth factor-beta modulates the expression of osteoblast and chondroblast phenotypes *in vitro*. *J Cell Physiol* 134:337–346.

149. Rotter N, Sittinger M, Hammer C, Bujía J, Kastenbauer E. (1997). Transplantation *in vitro* hergestellter Knorpelmaterilien:Charakterisierung der Matrixsynthese. *Laryngo Rhino Otol* 76:241–247.

150. 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. *Biomed Mater Res* 42:347–356.

151. Rotter N, Ziegelaar BW, Staudenmaier R, Happ T, Naumann, Kastenbauer E. (2001). Charakterisierung humaner respiratorischer Epithelzellen auf resorbierbaren Biomaterialien. *HNO Informationen* 2:170.

152. Sabatini LM, Allen-Hoffmann BL, Warner TF, Azen EA. (1991). Serial cultivation of epithelial cells from human and macaque salivary glands. *In Vitro Cell Dev Biol* 27A:939–948.

153. Sachs BL, Goldberg VM, Moskowitz RW, Malemud CJ. (1982). Response of articular chondrocytes to pituary fibroblast growth factor (FGF). *J Cell Physiol* 112:51–59.

154. Sanan AS, Haines SJ. (1997). Repairing holes in the head:A history of cranioplasty. *Neurosurgery* 40:588–603.

155. Scheffler B, Raeber G, Mayer J, Kotte BM, Bootz F, Wintermantel E. (2000). Human bone cells on non degradable scaffolds—An in vitro study. *Cells Tissues Organs* 166:111.

156. Schick B, Hendus J, Abd El Rahman EL Tahan, Draf W. (1998). Rekonstruktion der Stirnregion mit Tabula externa des Schädels. *Laryngo Rhino Otol* 77:474–479.

157. Schwarz RP, Goodwin TJ, Wolf DA. (1992). Cell culture for three-dimensional modeling in rotating-wall vessels:An application of simulated microgravity. *J Tissue Cult Methods* 14:51–58.

158. Sens DA, Hintz DS, Rudisill MT, Sens MA, Spicer SS. (1985). Methods in laboratory investigation—Explant culture of human submandibular gland epithelial cells: Evidence for ductal origin. *Lab Invest* 52:559–568.

159. Shirasuna K, Sato M, Miyazaki T. (1981). A neoplastic epithelial

duct cell line established from an irradiated human salivary gland. *Cancer* 48:745–752.

160. Sittinger M, Buija 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.

161. Sittinger M, Bräunling J, Kastenbauer E, Hammer C, Burmester G, Bujía J. (1997). Untersuchungen zum Vermehrungspotential von Nasenseptum-Chondrozyten für die in vitro-Züchtung von Knorpeltransplantaten. *Laryngo Rhino Otol* 76:96–100.

162. Sittinger M, Tánczos E. (2001). Tissue Engineering—Hoffnungsträger der regenerativen Medizin. *transkript Laborwelt* 1:12–16.

163. Strome M, Stein J, Esclamado R, et al. (2000). Laryngeal transplantation and 40–month follow-up. *N Engl J Med* 344:1676–1679.

164. Stuart CA, Furlanetto RW, Lebowitz HE. (1979). The insulin receptor of embryonic chicken cartilage. *Endocrinology* 105:1293–1305.

165. Szmeja Z, Szyfter W, Wojtowicz JG, Kruk-Zagajewska A. (1999). Extendend fronto-lateral laryngectomy with simultaneous reconstruction by means of a mucochondral nasal septum flap. *Eur Arch Otorhinolaryngol* 256:390–391.

166. Terheyden H, Jepsen S, Rueger DR. (1999). Mandibular reconstruction in miniature pigs with prefabricated vascularized bone grafts using recombinant human osteogenic protein-1: A preliminary study. *Int J Oral Maxillofacial Surg* 28:461–463.

167. Terheyden H, Jepsen S, Möller B, Rueger D. (2001). Sinusbodenaugmentation mit simultaner Implantatinsertion unter Verwendung von rekombinantem humanem Osteogenic Protein-1. *Laryngo Rhino Otol* 80:47–51.

168. Terheyden H. Personal communication, 2001.

169. Thompson AY, Piez KA, Seydin SM. (1985). Chondrogenesis in agarose gel culture. *Exp Cell Res* 157:483–494.

170. Thomson HG, Kim T, Ein SHb. (1995). Residual problems in chest donorsites after microtia reconstruction. *Plast Reconstr Surg* 95:961–968.

171. Trippel SB. (1995). Growth factor actions on articular cartilage. *J Rheumatol Suppl* 43:129–132.

172. Ueda M, Ebata K, Kaneda T. (1991). *in vitro* fabrication of bioartifical mucosa for reconstruction of oral mucosa:Basic research and clinical application. *Ann Plast Surg* 27:540–549.

173. Urist MR. (1965). Bone:Formation by autoinduction. *Science* 150:893–899.

174. Urist MR, Granstein R, Nogami H, Svenson L, Murphy R. (1977). Transmembrane bone morphogenesis across multiple-walled diffusion chambers. *Arch Surg* 112:612–619.

175. Vacanti CA, Langer R, Schloo B, Vacanti JP. (1991). Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. *Plast Reconstr Surg* 88:753–759.

176. Vacanti CA, Cima LG, Ratkowski D. (1992). Tissue engineering growth of new cartilage in the shape of a human ear using synthetic polymers seeded with chondrocytes. *Mater Res Soc Symp Proc* 252:367–374.

177. Vacanti CA, Paige KT, Kim WS, Sakata J, Upton J, Vacanti JP. (1994). Experimental tracheal replacement using tissue engineered cartilage. *J Pediatr Surg* 29:201–205.

178. Vacanti CA, Vacanti JP. (2000). The science of tissue engineering. *Orthop Clin North Am* 31:351–355.

179. Vacanti JP, Langer R. (1999). Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation. *Lancet* 354(Suppl I):32–34.

180. Vivien D, Galera P, Lebrun E, Loyau G, Pujol JP. (1990). Differential effects of transforming growth factor-beta and epidermal growth factor on the cell cycle of cultured rabbit articular chondrocytes. *J Cell Physiol* 143:534–545.

181. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. (1993). Head and neck cancer. *N Engl J Med* 328:184.

182. Von der Mark K, Gauss B, von der Mark H, Müller P. (1977). Relationship between shape and type of collagen synthesized as chondrocytes lose their cartilage pheonotype in culture. *Nature* 267:531–532.

183. Wehmöller M, Eufinger H, Kruse D, Massberg W. (1995). CAD by processing of computed tomography data and CAM of individually designed prostheses. *Int J Oral Maxillofac Surg* 24:90–97.

184. Werntz JR, Lane JM, Burstein AH, Justin R, Klein R, Tomin E. (1996). Qualitative and quantitative analysis of orthotopic bone regeneration by marrow. *J Orthop Res* 14:85–93.

185. Wickware P. (2000). Exploring the territory in tissue engineering. *Nature* 403:464–465.

186. Williams DF, ed. (1987). Definitions in biomaterials. In: *Proceedings of a Consensus Conference of the European Society for Biomaterials, Chester, England, 1986. Progress in Biomedical Engineering.* Amsterdam: Elsevier, pp. 65–71.

187. Wintermantel E, Ha SW. (1996). Biokompatible Werkstoffe und Bauweisen:Implantate für Medizin und Umwelt. Berlin: Springer-Verlag.

188. Wintermantel E, Mayer J, Blum J, Eckert KL, Luscher P, Mathey M. (1996). Tissue engineering using superstructures. Biomaterials 17:301–310.

189. Wintermantel E, Mayer J, Ruffieux K, Bruinink A, Eckert KL. (1999). Biomaterialien—humane Toleranz und Integration. *Chirurg* 70:847–857.

190. Wolff D, Goldberg VM, Stevenson S. (1994). Histomorphological analysis of the repair of the segmental diaphyseal defect with ceramic and titanium fibermetal implants:Effects of bone marrow. *J Orthop Res* 12:439–446.

191. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA. (1988). Novel regulators of bone formation: Molecular clones and activities. *Science* 242:1528–1534.

192. Wu R, Yankasas J, Cheng E, Knowles M, Boucher R. (1984). Growth and differentiation of human nasal epithelial cells in culture. Serum-free, hormone supplemented medium and proteoglycan synthesis. *Am Rev Respir Dis* 132:311–20.

193. Wu R. (1985). *In vitro* differentiation of airway cells. In: Schiff LJ, ed. *In Vitro* Models of Respiratory Epithelium. Boca Raton, FL: CRC Press, pp. 1–26.

194. Zimber MP, Tong B, Dunkelman N, Pavelec R, Grande D, New L, Purchio AF. (1995). TGF-$\beta$ promotes the growth of bovine chondrocytes in monolayer culture and the formation of cartilage tissue on three-dimensional scaffolds. *Tissue Eng* 1:289–300.

195. Zingg AE, Froesch ER. (1973). Effects on partially puriefied preparations with non-suppressible insulin-like activity on sulfate incorporation into rat and chicken cartilge. *Diabetologia* 9:472–476.

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