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Cartilage and bone tissue engineering for reconstructive head and neck surgery

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Abstract The loss of cartilage and bone because of congential defects, trauma and after tumor resection is a major clinical problem in head and neck surgery. The most prevalent methods of tissue repair are through autologous grafting or using implants. Tissue engineering applies the principles of engineering and life sciences in order to create bioartificial cartilage and bone. Most strategies for cartilage tissue engineering are based on resorbable biomaterials as temporary scaffolds for chondrocytes or precursor cells. Clinical application of tissue-engineered cartilage for reconstructive head and neck surgery as opposed to orthopedic applications has not been well established. While in orthopedic and trauma surgery engineered constructs or autologous chondrocytes are placed in the immunoprivileged region of joints, the subcutaneous transplant site in the head and neck can lead to strong inflammatory reactions and resorption of the bioartificial cartilage. Encapsulation of the engineered cartilage and modulation of the local immune response are potential strategies to overcome these limitations. In bone tissue engineering the combination of osteoconductive matrices, osteoinductive proteins such as bone morphogenetic proteins and osteogenic progenitor cells from the bone marrow or osteoblasts from bone biopsies offer a variety of tools for bone reconstruction in the craniofacial area. The utility of each technique is site dependent. Osteoconductive approaches are limited in that they merely create a favorable environment for bone formation, but do not play an active role in the recruitment of cells to the defect. Delivery of inductive signals from a scaffold can incite cells to migrate into a defect and control the progression of bone formation. Rapid osteoid matrix production in the defect site is best accomplished by using osteoblasts or progenitor cells.

Keywords Tissue engineering · Biomaterials · Cell culture · Cartilage · Bone · Stem cells

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Introduction

The most common need for cartilage and bone in the head and neck area is for reconstruction of the nose and the ears or bone of the craniofacial region to correct congenital deformities or to replace tissue after trauma or tumor resection. Autologous cartilage and bone are limited in their supply and require additional invasive surgical procedures. A possible future alternative to obtain tissue for reconstructive head and neck surgery is to generate autologous cartilage and bone in vitro with the help of tissue engineering. Tissue engineering is the combination of in vitro engineered cells, tissues and molecules with one or several biomaterials to reconstruct a defect or function in an organism [29].

Cartilage

Cartilage lacks an intrinsic regeneration capacity. Therefore cartilage defects need to be reconstructed with transplants. Today, autologous cartilage is the gold standard for plastic and reconstructive surgery of the nose and auricle (Fig. 1) [40]. Cartilage is excised from the rib, the auricle or the nasal septum [34, 47]. Excision of cartilage, however, requires a second surgical procedure with additional donor site morbidity, such as, e.g.,

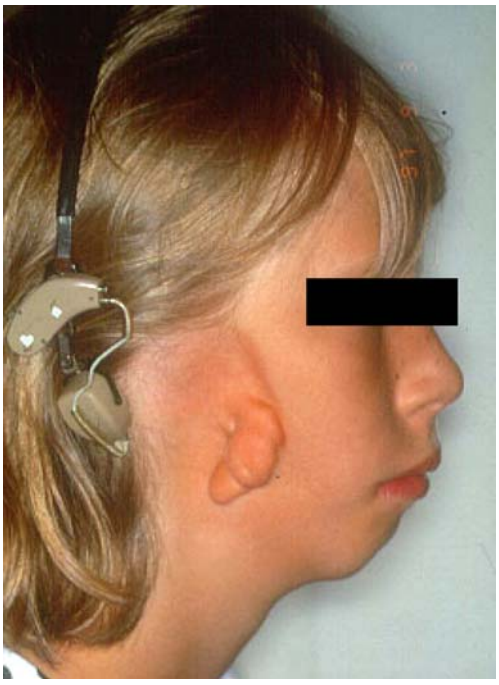

Fig. 1 Congenital microtia with associated atresia of the external and middle ear. For autologous reconstruction cartilage has to be harvested from the ribs inducing a secondary surgical trauma to the patient

wound infections, insufficient cosmetic results at the donor site and postoperative pain. As the amount of cartilage donor tissue available for these procedures is limited, cartilage has become one main target in tissue engineering research.

Most strategies for cartilage tissue engineering are based on resorbable biomaterials as temporary scaffolds for chondrocytes or precursor cells [58]. Cells are amplified in vitro, seeded onto the scaffold and then transplanted. Differentiated cells produce cartilage-specific matrix components in vivo. The developing tissue should resemble native cartilage with regard to the specific function and morphology.

Cartilage cell culture and scaffold design

For most tissue-engineering procedures in the head and neck, native cartilage has been derived from septo- or septorhinoplasty [18, 50, 53]. After freeing the cartilage from any adjacent tissue, it is mechanically cut into pieces about 1×1-mm in size. These pieces of cartilage are enzymatically digested for 12 to 18 h. This procedure leads to a single cell chondrocyte solution. Sittinger et al. were able to isolate about 0.5×10^6 from a 0.5 cm^3 cartilage specimen with a cell vitality higher than 85%. From this initial isolation cells were amplified up to 1,000 to 100,000 times within 8 weeks [54].

Strong in vitro amplification results in the loss of tissue-specific functions, a process called dedifferentiation [61]. However, to engineer functional tissue in vitro, cells have to maintain their specific functions. A basic knowledge about mechanisms of amplification and dedifferentiation of human chondrocytes is therefore of utmost concern for tissue engineering of cartilage.

After several days in monolayer culture chondrocytes develop a fibroblast-like type of shape with cellular processes, which contain filaments of actin derived from the cytoskeleton forming stress fibers [31]. Chondrocytes discontinue the synthesis of cartilage-specific collagen type II and chondroitin-4-sulfate [61] and start to synthesize collagen types I and III instead [2, 13, 32]. In this state of dedifferentiation chondrocytes start to proliferate. It is possible to increase their proliferative capacity by adding certain factors, such as fetal calf serum or suitable growth factors.

Culture conditions enabeling a three-dimensional arrangement of chondrocytes such as, e.g., agarose or alginate gels enable chondrocyte redifferentiation. During redifferentiation the cells regain their typical round shape and restart the synthesis of cartilage-specific collagen type II and of cartilage-specific proteoglycans [2, 3]. Differentiation and the degree of differentiation are also influenced by the initial cell number in monolayer culture via autocrine stimulation mechanisms [24]. From these cellular characteristics it becomes clear that one of the most important prerequisites for tissue engineering of cartilage is redifferentiation of amplified chondrocytes. In tissue engineering redifferentiation can be guided by the porosity and structure of the three-dimensional scaffold. The porosity of native cartilage is about 78% [1]. Highly porous scaffolds closely mimic this structure and allow for maximal adhesion and proliferation of seeded chondrocytes, while leaving space for newly synthesized matrix via a large surface-volume-ratio. Since cartilage is avascular, cartilage nutrition and metabolism is mainly influenced by local diffusion and cell-matrix interactions. The scaffold should not inhibit diffusion, which is especially relevant when culturing large amounts of cells for transplants, as necessary for clinical application.

The mechanical properties of the scaffold should resist physiologic forces at the implantation site until the newly formed matrix is strong enough to resist these forces itself. The elastic modulus of native cartilage is about 0.79 MPa; the shear modulus is about 0.68 MPa [1]. Recent investigations have demonstrated that biomechanical properties of tissue-engineered human cartilage closely resemble these data [17].

Engineering cartilage transplants in vitro

Until now, engineering of cartilage constructs in vitro mainly has been achieved by the seeding of chondrocytes onto biodegradable polymer scaffolds. Early experimental work published by Vacanti and Puelacher and their co-workers in 1992 and 1994 demonstrated relevant approaches for clinical tissue engineering in the

head and neck. Constructs in the shape of a human ear [59], the trachea [60], the temporomandibular joint and the nasal septum [44, 45] were first investigated by this group. Chondrocytes were isolated from bovine articular cartilage. Rotter et al. and Haisch et al. were able to demonstrate that tissue engineering of clinically relevant bioartificial cartilage is possible with human chondrocytes [18, 49] (Fig. 2a,b). The common variable of the above investigations is that in vitro seeded scaffolds were transplanted into a nude mouse model, where further maturation of the constructs into cartilage took place.

However, until now the clinical application of tissue-engineered cartilage for the head and neck as opposed to orthopedic applications has not been well established. This results from the different implantation site and the different requirements concerning the shape and initial mechanical strength. While in orthopedic surgery engineered constructs are placed in the immunoprivileged region of the joints [6], the subcutaneous transplant site in the head and neck can lead to strong inflammatory reactions and resorptions. Another difference is the shape of the transplants, which needs to be well defined before transplantation in the head and neck, whereas defects in joints are usually preformed "holes" that can easily be filled by liquid or semisolid cell preparations.

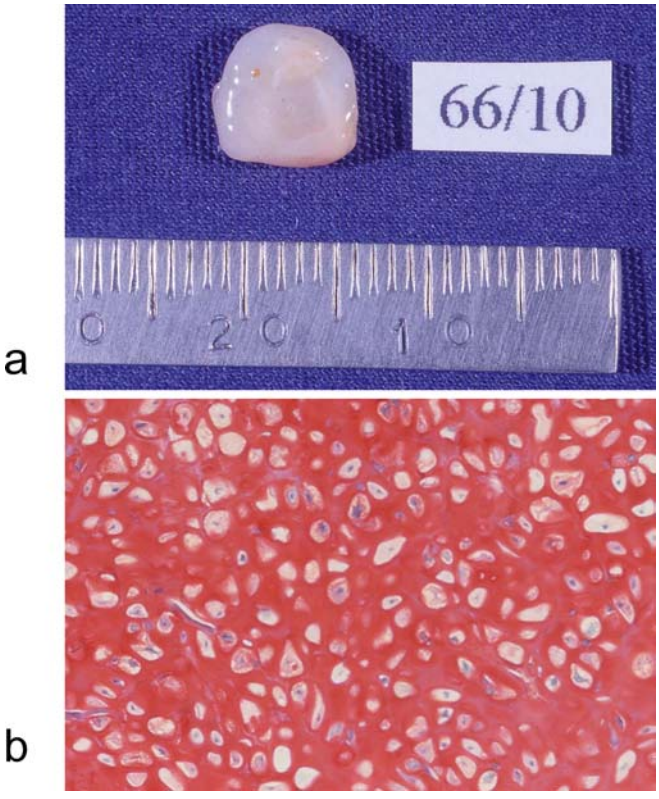

Fig. 2 a, b Macroscopic and histological aspect of tissue-engineered cartilage. The cartilage is clearly seen containing chondrocytes in lacunae surrounded by cartilaginous matrix that takes up the red safranin O stain (**b**). Original magnification ×125

First clinical results

In 1997 one patient received an tissue-engineered auricle at the Department of Otolaryngology at Charité University in Berlin, Germany (Fig. 3). Details of the in vitro-procedures are described earlier in this journal [20]. The engineered cartilage remained stable in shape for about 3 weeks, but was strongly resorbed afterwards, leading to an unfavorable cosmetic result. In 2000 the Department of Plastic and Hand Surgery at the University of Freiburg, Germany, reported the treatment of traumatic partial ear defect with tissue-engineered cartilage derived from costal chondrocytes [41]. This effort also failed as the construct was completely resorbed after a few months.

So far, it is impossible to protect the neocartilage in subcutaneous transplantation locations from rejection and resorption in a sufficient manner. Physiological wound healing leads to cellular reactions mainly directed by macrophages. Autoantibodies directed against different types of collagen [11, 36] might be responsible for these reactions. Encapsulation of tissue-engineered cartilage might be an alternative to protect transplants from resorption and to act as an immunological barrier. Dautzenberg et al. first described a method of tissue encapsulation with natriumcellulosesulfate (NaCs) and polydialylldimethylammoniumchloride (PDADMAC) in 1996. Both substances spontaneously form membranes, which were shown to be mechanically stable and biocompatible in vivo [15, 16]. Haisch first applied this

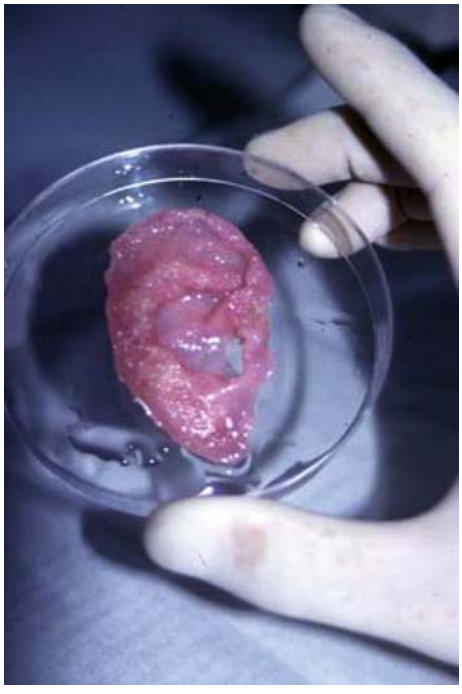

Fig. 3 Tissue-engineered auricle fabricated by using autologous chondrocytes, fibrin gels and a resorbable polymer scaffold

method of macroencapsulation in native human cartilage transplants in a nude mouse model. While there were considerable inflammatory reactions in non-treated cartilage transplants, encapsulated cartilage only showed a minor inflammatory reaction in the encapsulation membrane. Resorption only took place in areas where there were tears in the polyelectrolytemembrane. Encapsulation using NaCs/PDADMAC membranes therefore seems to be a promising pathway to prevent postoperative resorption of tissue-engineered cartilage in the head and neck [19].

Bone

Other than cartilage, bone possesses an intrinsic repair capacity. Nevertheless, the fast surgical replacement of bone in the head and neck can become necessary because of defects caused by tumor resection (Fig. 4a, b), trauma, inflammation and congenital disorders. Today, autologous bone transplants, e.g., from the tabula externa or the iliac crest, are used frequently for the replacement of bone defects in the face and the skull [51]. Autogenous bone transplants require a second procedure to harvest tissue, which might lead to additional surgical complications, such as unfavorable scar formation and recurrent pain [22]. During harvesting, bone transplants from the bony skull seroma, hematoma, insufficient wound healing, lesions of the dura, surbarachnoidal bleeding, lesions of the sagittal sinus and intracerebral bleeding can occur [43, 52]. Tissue engineering of the bone might combine the advantages of autologous bone transplants with a reduction of secondary harvesting operations. Three main strategies may be used alone or in combination for the replacement and regeneration of bone: matrix-based therapy, factor-based therapy and cell-based therapy.

Matrix-based bone replacement

Matrix-based therapies require implants that most closely replace missing bony structures. After implantation, osteogenic precursor cells and osteoblasts are required to migrate into the artificial matrix. This process is called osteoconduction [12]. The development of ceramic-based scaffolds such as hydroxyapatite and tricalcium-phosphate lead to the clinical application of osteoconduction for the regeneration of bone [10, 23]. The porous structure of the implants facilitates migration of cells and their three-dimensional arrangement. The main disadvantage is the lack of biological activity of the implant, such as, e.g., osteoinduction, which itself is the main goal of factor-based therapeutic strategies.

Factor-based bone replacement

Urist in the 1960s was able to show that reproducible intramuscular (heterotopic) osteoinduction by bone implants is only possible after complete demineralization of the bone [56]. Inside a bony defect the partial or complete decalcification of bone enables the diffusion of osteoinductive proteins from the adjacent bone matrix to initiate cellular differentiation in the surrounding tissue [37, 57]. In the late 1980s, Wozney and co-workers were able to characterize, isolate and clone for the first time some of the important bone morphogenetic proteins (BMPs) from bone [63]. BMPs are usually classified in the TGF-β family because of their amino acid structure [14, 38]. BMPs induce chemotaxis of perivascular, undifferentiated mesenchymal cells in soft tissue and in undifferentiated stem cells in the bone marrow by binding to specific serin-threonin-kinase-receptor complexes on their membrane surface [46]. The increase of receptor binding leads to the proliferation of these cells with concurrent differentiation into cartilage and bone precursor cells. During enchondral ossification, bone

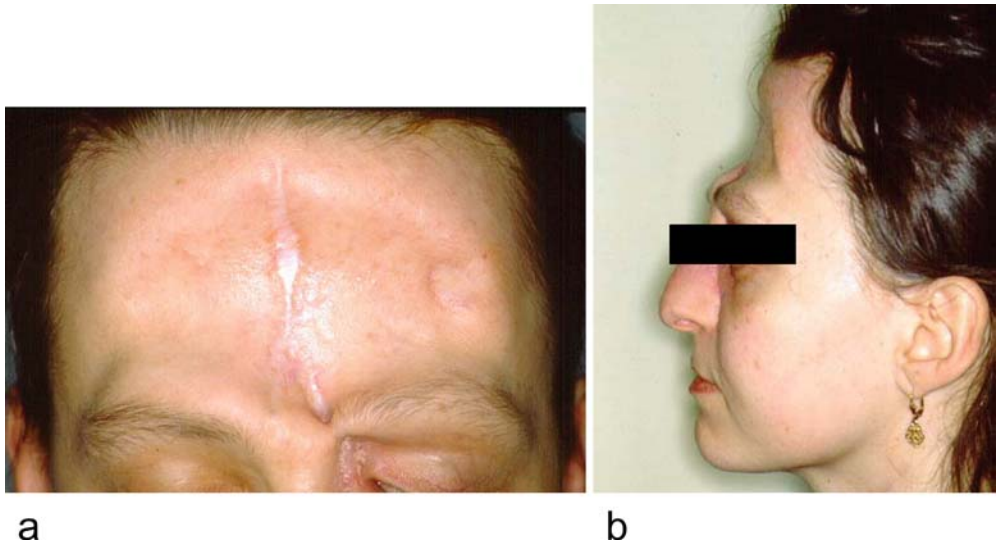

Fig. 4 a, b Cranial defect after resection and radiation therapy of a malignant schwannoma of the anterior skull base

tissue containing bone marrow develops within a few days. The application of BMPs for the replacement of bone is a promising pathway. Bone induction by BMPs can be ortotopical as well as heterotopical, such as, for example., inside muscular tissue. This property reveals the possibility of intramuscular bone induction by injection of BMP to transplant it with the help of a free or pedicled muscular flap [27, 55].

One basic requirement for the clinical application of osteoinductive proteins is the development of suitable delivery systems and scaffolds. Depending on the site of the implant, biomechanical requirements have to be met. Investigations using *E. coli* derived BMP-2 were able to demonstrate that there is osteoinduction in combination with almost every commercially available bone replacement material [28, 48].

Cell-based bone replacement

The cell-based therapy concept was first established using fresh autologous bone marrow [25, 39, 62]. Bone marrow contains osteogenic progenitor cells with the potential to induce bone regeneration [4]. In the clinical application, bone marrow was derived from the iliac crest and directly applied to the bone defect. For tissue-engineering bone marrow precursor cells of bone as well as differentiated osteoblasts or periosteal cells might be combined with biomaterials like demineralized bone matrix.

Osteoblasts can be either derived from bone biopsies, precursor cells from the bone marrow or the periosteum and can be differentiated into osteoblasts in vitro, e.g., by adding dexamethasone and ascorbic acid [7]. Similar to the concept used in cartilage tissue engineering, osteoblasts are seeded onto biocompatible scaffolds in vitro (Fig. 5a, b). In an animal model, differentiated osteoblasts derived from precursor cells were seeded onto a polyglycolic acid scaffold to treat a full thickness calivarian defect. There was clear evidence of new bone formation 12 weeks after implantation [5].

Stem cell-based tissue engineering

Recently, the successful isolation of human stem cells from bone marrow, periosteum and fat tissue was established by different groups [9, 21, 35, 42]. These cells are highly proliferative and are capable of differentiating into different types of tissue such as bone, cartilage, tendon, muscle or fat. Human mesenchymal stem cells are characterized by a specific pattern of cell surface markers, growth factors, cytokine receptors, integrins and other adhesion molecules [8, 42]. Today, it is possible to amplify mesenchymal stem cells from an adult human for over 30 passages in vitro, thus obtaining more than 1 billion cells. During amplification, cells maintain their phenotypic characteristics without loosing their differentiation capacity, e.g., the osteogenic or chondrogenic potential [7, 42]. In experiments using a rat and a rabbit model, mesenchymal stem cells were seeded onto hydroxyapatite and then used to repair femur defects. The group treated with mesenchymal stem cells showed formation of neobone and better mechanical characteristics than the group treated without cells. The cell free group was well vascularized without any remarkable bone formation [8, 26].

For the potential clinical application of tissue-engineered tissues, differentiated cells, precursor cells or mesenchymal stem cells might be used alone or in

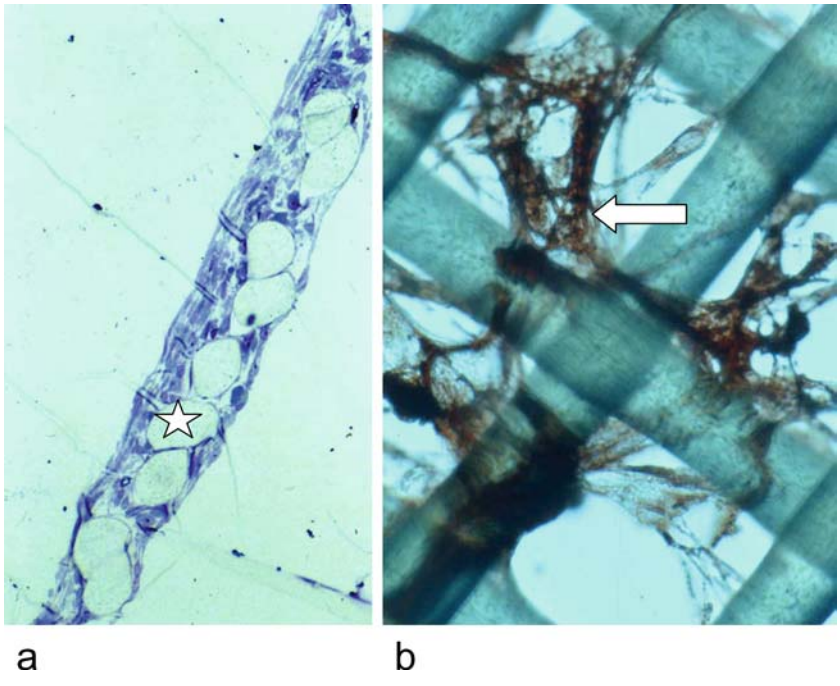

Fig. 5 a, b Histological and immunocytochemical investigation of a polyethylentherephtalate (PET)-scaffold seeded with human osteoblast-like cells from the nasal septum. The single fibers of the scaffold (*white star*) are surrounded by cells and matrix (**a**). HE, Original magnification ×200. Positivity for osteocalcin (*white arrow*) indicates osteoblast-like-differentiation of the cells on the scaffold (**b**) Original magnification ×400

combination. So far, it is not known which cell type is best suitable for tissue engineering of different types of tissue in the head and neck.

Conclusions and perspectives

Tissue engineering enables the fabrication of living and functional tissue transplants, hence it is a possible alternative to classic surgical reconstruction techniques. Before clinical success can be achieved in reconstructive head and neck surgery, several problems remain to be solved. Until today, only a minor part of all somatic organ-specific cell types can be amplified in vitro to a sufficient extent with the perspective to redifferentiate these cells afterwards.

The availability, isolation and propagation of human stem cells will most likely be a main factor for tissue engineering in the future [30]. Whether totipotent or pluripotent adult precursor cells or even embryonic stem cells will be available for tissue engineering procedures in the future will depend on research progress as well as on the political and ethical decisions taken by society. The combination of genetic engineering with tissue engineering, such as, for example, the development of immortalized cells will open additional possibilities for cell-based therapies [33].

Sufficient supplies with oxygen and nutrients are limiting factors in the engineering of complex tissues and organs. Even though complex culture systems are able to supply complex composite tissues with adequate amount of nutrients in vitro, this amount needs to be maintained continuously in vivo. The survival of tissue without capillary vessels is questionable.

Characterization of the optimal scaffold for different applications—different types of tissue and localizations of the implant—remains to be investigated. Whether the encapsulation of in vitro engineered tissue with polyelectrolytmembranes will still be efficient after connection to the local vascular network and will still protect the implant from local and system immunological reactions remains to be studied.

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