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Review Article

Polymeric implant materials for the reconstruction of tracheal and pharyngeal mucosal defects in head and neck surgery

Abstract

The existing therapeutical options for the tracheal and pharyngeal reconstruction by use of implant materials are described. Inspite of a multitude of options and the availability of very different materials none of these methods applied for tracheal reconstruction were successfully introduced into the clinical routine. Essential problems are insufficiencies of anastomoses, stenoses, lack of mucociliary clearance and vascularisation. The advances in Tissue Engineering (TE) offer new therapeutical options also in the field of the reconstructive surgery of the trachea. In pharyngeal reconstruction far reaching developments cannot be recognized at the moment which would allow to give a prognosis of their success in clinical application. A new polymeric implant material consisting of multiblock copolymers was applied in our own work which was regarded as a promising material for the reconstruction of the upper aerodigestive tract (ADT) due to its physicochemical characteristics. In order to test this material for applications in the ADT under extreme chemical, enzymatical, bacterial and mechanical conditions we applied it for the reconstruction of a complete defect of the gastric wall in an animal model. In none of the animals tested either gastrointestinal complications or negative systemic events occurred, however, there was a multilayered regeneration of the gastric wall implying a regular structured mucosa.
In future the advanced stem cell technology will allow further progress in the reconstruction of different kind of tissues also in the field of head and neck surgery following the principles of Tissue Engineering.

Keywords: upper aerodigestive tract, multifunctional polymer implant materials, pharynx reconstruction, tracheal reconstruction, tissue engineering, gastric wall regeneration

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1 Introduction

1.1 History of implant materials

Body foreign materials were used for medical purposes already in the ancient times. In Egypt linen soaked with rubber were used for wound closure. Sculls also dating back to ancient times with gold inlays in teeth were found in Ecuador. Descriptions of urological catheters exist from the Romans. Arm-, hand-, leg- and food prostheses were developed during the Renaissance. The missing knowledge, however, with respect to the importance of sterility and sterilization was the biggest problem until the second halve of the 19th century because infections endangered the implantations.
The 20th century can be called the era of synthetical polymers. In 1920 Staudinger published his idea of big macro molecules (polymers) built up from smaller substructures (monomers). Poly(methylmethacrylat) (PMMA) was introduced in dental medicine in 1937 although the advantageous characteristics of PMMA implants became first known through war wounded pilots in World War II: Soft tissue and eye injuries induced by and containing small fractions of bursting airplane cockpits (PMMA) led to minute foreign body reactions only. Szilagyi et al. reported first clinical experiences with Poly(ethylenterephthalate) (trade name: Dacron®) as vascular arterial prostheses in 1958 [1]. In the 60th of the last century, Dr. John Charnley, an orthopedic surgeon from U.K. developed a functional and cemented total hip endoprosthesis based on steel and ultra high molecular weight polyethylene inlays which were cemented into the femoral bone using PMMA as “cement”. Beginning at the end of the 90th of the last century there was a focus on the development of degradable polymeric implant materials.
Since then the required profile of a biomaterial was more and more adapted to its application. The availability of so called polymer systems allows a large scale variation of material characteristics like mechanical performance and hydrolytic degradation and thus to adapt these ma-

terials to specific local requirements in the organism. Now even the functionality of implant materials was extended so that cellular and biological processes can now be assessed and influenced on site. The technological potential of these new implant materials is enormous and it is assumed that they will potentiate the development of new therapeutical options in medicine [2].

1.2 Regenerative Medicine

Due to the shift in morbidity during the last decades and the modern demographic development in the western world the clinical medicine has to deal more and more with diseases gradually leading to a loss of function of important cell and organ systems. In many cases these diseases cannot be cured by the currently available therapies and these patients have to remain in permanent therapy causing high costs. New therapies will hopefully be developed for many of these diseases by the Regenerative Medicine. Comprised are many of the wide spread diseases with high morbidity, serious reduction of life quality and high costs for the public health system. Problem solutions offered by the Regenerative Medicine are expected to offer a fully functional replacement of destroyed or damaged tissues so that permanent symptomatic therapies become dispensable in the interest of both, the patient and the public health system.

Regenerative Medicine is highly interdisciplinary and deals with the restitution, substitution, regeneration of non functional or more or less functionally impaired cells, tissues, organs through biological replacement, e.g. through tissues produced in vitro or through the stimulation of the body's own regeneration and/or repair processes [3], [4], [5]. This area is focused not only on organ and tissue replacement (Tissue Engineering) but comprises also new therapeutical options and further developments in classical transplantation medicine and cell therapies including the stem cell technology [6], [7]. Implied, too, are pharmacological options to be developed and aimed at the specific stimulated regeneration of tissues/organs [8]. All the actions recently started in this area are aimed to translate the results from research and development into new therapeutical options for the clinical medicine [9], [10], [11].

Important clinical success in stem cell research [12], [13] and the extracorporeal growth in bioreactors of cartilage, muscle and vascular endothelial tissues for transplantation purposes show the extensive potential of Regenerative Medicine [14], [15], [16], [17], [18]. The euphoric visions to grow complete and functional organs in vitro right now, however, were recognized to be very premature and led to the understanding that there are still serious deficits in our knowledge of regeneration in organisms. This implies basic research in the fields of cell- and developmental biology, of differentiation of cells and growth and maturation of tissues and organs, of immunological tolerance, of tissue and organ regeneration and wound healing, of stem cell development, of the development of multifunctional implant materials and of functional molecular imaging. In the field of applied research innovative solutions are needed to couple technical systems to the organism. This is necessary for adapted bioreactor systems as well as for drug delivery-systems with biosensor regulated local release of biomolecules and also, especially, for the non-invasive assessment and continuous validation of the local success of regenerative processes.

Nationally and internationally the area of Regenerative Medicine is characterized by an outstanding speed of progress. A high number of different fields of science and technology have to be combined and united to arrive at innovative problem solutions. Since 1990 the German Ministry for Research and Education (BMBF) and German Research Council (DFG) spent 230,000,000 Euro for basic research and translation in the area of Regenerative Medicine [19]. The rate of publications in Regenerative Medicine increased exponentially in the last years. The annual number of publications meanwhile amounts to 9000 (August 2008). However, the participation of clinicians is necessary right from the beginning of the development of new therapeutical options. Clinicians, especially, recognize the necessity of innovative therapeutic developments and their adaptation to the clinical practice. A major hurdle in the clinical establishment of new therapies based on Regenerative Medicine is thought to be the financial aspect [20], [21]. A study published by the BMBF (April 2007) recognized the practice of reimbursement by the health insurance companies as the biggest obstacle for the development of products to be applied in clinical routine. Mentioned further in this study were the legal regulations of approval of medicinal products and of clinical studies. It was assumed that health insurance companies are willing to reimburse new products only after extensive and long-term studies [19].

From a clinical perspective further developmental obstacles result from complex legal regulations of e.g. transplants/embryonic stem cells in Europe and in Germany especially. As far as the Medical Preparations Act (Arzneimittelgesetz, AMG) will continue to be applied to tissues and tissue related products, very extensive and lengthy processes of approval for therapies based on extra corporeal growth of tissues in bioreactors will be afforded [22], [23], [24], [25]. According to the new German Tissue Law all cell containing parts of the human body which are no organs are defined as tissues where the AMG applies. The Federal Chamber of Physicians (Bundesärztekammer, BÄK) recognized further legal and practical obstacles that might endanger the supply with extracorporeally grown tissues in Germany. These are especially the definitions of institutions for the explantation of tissues and for the tissue processing as they are found in the Transplantation Law (Transplantationsgesetz, TPG) which was generated following an EU directive. These definitions are neither in concordance with the EU directive nor with the Medical Preparations Act (AMG).

1.3 Functionalized implant materials

The experiences with polymer implants used in medicine which will be described in the following with respect to their development and therapeutical applications led to clear requirement profiles for the future use of polymeric implant materials. The functionality of implant materials has to be broadened so that they are stimuli sensitive and e.g. change their physico-chemical behavior in answer to external stimuli or induced biological processes at the site of implantation. Bioactive substances like peptides, proteins or carbohydrates might be immobilized by polymers or released from implants in a well defined process. The most up-to-date trend in polymer sciences is the development of degradable biomaterials showing multifunctionality. This means that specific functionalities like e.g. hydrolytic degradation, physiological and biomechanical tissue compatibilities and shape memory can be adapted to the region specific requirements of the site of implantation [26], [27].
AB-Copolymernetworks are an example for an implant material which can be functionalized. These networks are produced by photocrosslinking of Oligo(ε-caprolacton)dimethacrylate and polybutylacrylate segments [28], [29]. The incorporation of flexible polybutylacrylate segments allows e.g. the tailoring of material elasticity which is an important condition for the biomechanical functionality of this polymer system in the temperature range between room and body temperature. AB-Copolymernetworks are biodegradable due to their hydrolytically cleavable polyester chain segments. Beside the hydrolysable ester bonds the polymer networks contain oligomethacrylate- and polybutylacrylate chains which cannot be cleaved hydrolytically. With the progressing hydrolytical degradation an increasing amount of oligomeres of methacryle acid and acryle acid derivatives is derived from these segments. These are water soluble and nontoxic in low doses [30]. In case of the AB-Copolymernetworks a high residual polymer weight is expected for the oligobutylacrylate derivatives. A molecular weight of 200,000 g mol-1 is discussed as critical upper limit for water soluble biostabile macromolecules in biomedical applications because there can be an accumulation in the blood circulation above this molecular weight limit [31]. Due to their degradability, stimuli sensitivity, biocompatibility and -functionality these copolymer networks are termed multifunctional. Biomechanical characteristics as well as types and periods of degradation can be adjusted as well.

1.4 Sterilization of polymer based degradable implant materials

The sterilization of implant materials is a precondition for their biomedical use. Polymer based and especially hydrolytically degradable biomaterials in general have a considerably lower thermal and chemical stability as ceramic or metallic materials. That is why they are generally not sterilized with conventional sterilization methods like heat sterilization (temperatures between 160–190 °C) or steam sterilization (121–134 °C) because otherwise the polymers could be damaged. Sterilization applying ionizing irradiation can change the chemical structure of polymers either by chain degradation or by new crosslinking of chains so that surface characteristics as well as thermal and mechanical bulk properties can be strongly influenced [32]. A change of the chemical surface structure of implant materials can influence their biocompatibility in vitro and in vivo [33]. Since the sterilization of polymer based biomaterials makes high demands on the sterilization method, low-temperature sterilization methods like plasma sterilization (low-temperature plasma sterilization, LTP) and ethyleneoxide (EO) sterilization are in the focus of intensive contemporary research [34], [35], [36], [37].

2 Regenerative Medicine for the reconstruction of the upper aerodigestive tract

Head and neck surgery is concerned with the reconstruction of damaged local tissues like mucosa, cartilage, bone or skin due to congenital anomalies, progressive diseases as well as therapeutical interventions. Fistulae of different genesis are associated with most serious complications in the head and neck area [38], [39], [40], [41]. These fistulae cause high rates of morbidity and mortality through the development of sepsis, pneumonia or bleeding from destruction of the carotid wall. The permanent secretion from fistulae and the cervical soft tissue defects especially of pharyngocutaneous fistulae is associated with a tremendous reduction of life quality of patients and their stigmatization [38]. Due to postoperative salivary fistulae in oncological patients their irradiation may not be possible within the planned periods so that therapeutical aims cannot be reached. Salivary fistulae are a relevant cost factor since the introduction of DRGs (Diagnosis Related Groups) in the german health system [42], [43]. Contemporary therapeutical options in the treatment of pharyngocutaneous fistulae depend on the size of fistulae and on the indication of a postoperative adjuvant irradiation therapy.

2.1 First applications of different implant materials in tracheal surgery

The modern tracheal surgery started with the first successful reanastomosis of the cervical trachea in dogs by Gluck and Zeller in 1881. First results of the tracheal reconstruction using implant materials were published by Daniel in 1948 [44]. Rigid tubes made of glass or metal were used for the defect reconstruction after the resection of tracheal segments (between 1 and 13 tracheal cartilage rings were excised) of dogs. These tubes had lips at the proximal and distal ends which were fixed by ligatures with the tracheal ends (Figure 1). The postoperative ob-

servation period lasted up to 6 months. In those animals surviving the surgery (14/15) extensive foreign bodies appeared after the embedding of implant materials in granulation tissues. There was also the development of dyspnoe. Daniel et al. published another study dealing with the reconstruction of tracheal and bronchus defects in dogs in 1950 [45]. While in the first publication the scientific focus was set on the surgical method, the authors dealt with functional aspects in the second one. The rates of healing or stenosis in tracheal reconstruction using rigid metallic prosthesis depended according to the authors on the anatomical localization: A higher success in reconstruction combined with lower rates of stenosis was found in the thoracic trachea than in the cervical trachea. In all experiments there was an extensive formation of granulation tissues around the implant materials with a consecutive tracheal stenosis. As further problems appeared the breakdown of sutures and infections [45]. In the 50ies a great number of experiments for the tracheal reconstruction was performed in animals using different materials like acrylresin [46], tantalum [47], stainless steel [48], polyethylene [49], nylon [50] and teflon [51]. The great number of materials used and the short survival time of the animals demonstrated that the problem of tracheal reconstruction using implant materials could not be solved at this time. The importance of biocompatibility of implant materials and the variable requirements depending on the implantation site became obvious at the end of the 50ies. After the successful application of Dacron® as arterial prosthesis (1958) it was realized that an appropriate material was not available for the tracheal reconstructive surgery showing the necessary elasticity, rigidity, and biocompatibility. At the end of the 50ies and the beginning 60ies there were first trials for the temporary application of polymeric implant materials in the tracheal reconstruction. These materials were covered with mucosa from the urinary or gall bladders to induce connective tissues or bone around tracheal stents. The temporary application meant that the implant material should be removed after the newly grown cartilage or bone in the former tracheal defect zone gave a sufficient stability so that the reconstructed tracheal tissues would not collapse. Although cartilage and bone tissues could be demonstrated histologically at the site of implantation, a sufficient tracheal stability could not be gained in any one of the animals and all animals died of respiratory insufficiency following tracheal obstruction after the removal of the differently coated implant materials [52], [53]. In the 60ies and 70ies further materials were tested for tracheal reconstruction e.g. Marlex networks (polyethylene/polypropylene networks) [54], silicon rubber [55] and Marlex networks covered with cartilage and/or tracheal mucosa [56], [57]. These new materials also did not fulfill the comprehensive requirements for tracheal reconstruction regarding mechanical strength and adequate flexibility to avoid vascular arrosion induced by mechanical irritation. These materials lacked biocompatibility, an air- and liquid tight integration of the implant materials into the adjacent body tissues, an adequate stability against bacterial invasion and, especially, the epithelialization of the implant with a functional tracheal epithelium [54], [55], [56], [57].

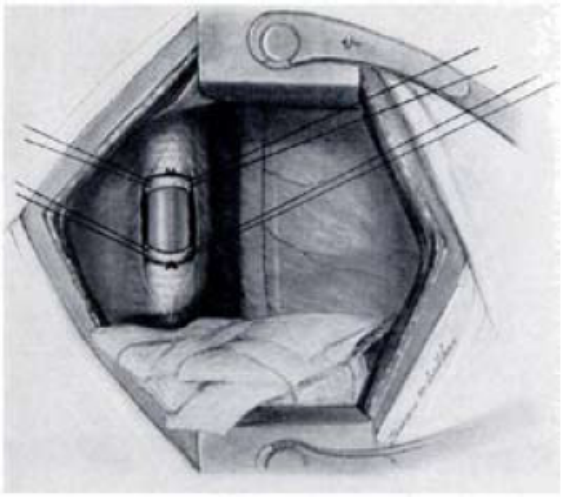

Figure 1: Method of fixation of rigid tubes of metal in the proximal and distal trachea of the tracheal defects in dogs with ligatures, from: Daniel RA, Taliaferro RM, Schaffarzick WR. Experimental Studies on the Repair of Wounds and Defects of the Trachea and Bronchi. Chest. 1950;17:427 [45].

Wenig et al. showed in 1987 that through application of a fibroblast collagen matrix for the tracheal reconstruction of circumscript defects the rate of tracheal stenosis could be reduced significantly [58]. In 1989, Schauwecker et al. demonstrated the importance of biomechanical properties of the implant materials depending on the site of implantation and that the porosity of the material surface was important for the integration of implants in surrounding tissues. These authors applied an isoelastic polyurethane prosthesis with different porosities at the luminal and abluminal surfaces for the reconstruction of 38 mm long defects of the cervical trachea of 19 dogs. Besides end-to-end anastomosis these authors applied inverted and everted techniques of anastomosis. The mean survival time of animals in case of the inverted technique was 27.7 days, in case of the everted technique 11.3 days and in case of the end-to-end anastomosis 19.5 days. The worst complications leading to a termination of these trials were local infections and insufficiencies of anastomosis in 12 of the animals and extensive stenoses accompanied by respiratory insufficiency in 7 animals. The authors observed that polyurethane prostheses with porous surfaces developed a tight integration into surrounding tissues but in none of the animals the luminal prosthetic surface was inhabited by a mucociliary epithelium. The authors attributed the high rate of complications primarily to the animal model chosen because the cervical mobility in dogs was said to be much higher than in humans, pigs or rats [59].

2.2 New methods and approaches for tracheal reconstruction

Key factors compromising the therapeutical success seem to be the regeneration of a functional mucociliary tracheal epithelium enabling the mucociliary clearance, foreign body reactions induced by implant materials, infections, and the necessity of reoperations in preoperated areas. The tissue engineering technique was described by Langer and Vacanti in 1993 and had 3 key components: cells for the tissue regeneration, polymer scaffolds as a matrix which support migration, proliferation and differentiation of cells as well as regulating factors which specifically influence the cellular behavior [60]. The following demands on tracheal prostheses were made: It should be a flexible but compression stable construct which is inhabited by a functional respiratory epithelium [61]. The complete epithelialization of prostheses is thought to be the main condition to allow an adequate mucociliary clearance and guarantee a reliable barrier against infection and invading connective tissue. There are still very few studies applying the methods of Tissue Engineering to produce tracheal replacements and to examine these in vitro and in vivo. Studies introduced by Vacanti et al. in 1994 were trend-setting where constructs based on Poly(glycolid) acid (PGA) and inhabited by bovine chondrocytes and tracheal epithelial cells were applied to close circumferential tracheal defects in rats [62]. In a consecutive study respiratory epithelial cells were isolated and injected into cartilage cylinders grown in vitro [63]. Examinations of these constructs revealed mature cartilage tissues as well as epithelial structures with a submucosal connective tissue. After 3 weeks in culture different stages of differentiation of a multilayered highly prismatic epithelium could be documented showing also some ciliary cells. In consecutive experiments these authors developed a tracheal replacement based on chondrocytes and fibroblasts which was implanted into sheep. The tracheal replacement thus generated could not be shown to develop kinocilia within the respiratory epithelial cells and therefore was not fully functional [64].

Besides the use of different implant materials in experimental and clinical trials throughout the last 50 years [45], [46], [47], [48], [49] there were many other attempts with autologous or allogenic tissues of different origin like fasciae, skin, bone and periost, cartilage and perichondrium, muscle, esophagus, pericardium, intestine and dura mater [65], [66], [67], [68], [69]. Again, high rates of complications were reported e.g. high rates of stenosis and necrosis, of anastomotic insufficiencies and a lack of mucociliary clearance.

At the end of the 90ies and the beginning of 2000 biodegradable stents were introduced in reconstructive tracheal surgery. Lochbihler et al. described in 1997 for the first time the application of a resorbable intratracheal stent made of polyglactine 910 filaments copolymerized with polydioxanone for the temporary stabilization of a tracheal stenosis in rats [70]. Korpela et al. applied a spirally shaped and reinforced stent made of poly(L-lactid) (PLA) to brigde tracheal stenoses in an animal model [71], [72]. Robey et al. described in 2000 the application of a biodegradable poly(L-lactid-co-glycolid) (PLGA) stent for the endotracheal stabilization of reconstructed circumscript defects in the anterior tracheal wall of rabbits using the faszia lata. Stenoses in those animals receiving intratracheal resorbable stents were significantly smaller than those in animals without stents. The high mortality rates of 17% in the implant group and 23% in the control group were mainly caused by the functionally relevant tracheal stenoses. This was the reason why the approach combining the use of autologous materials and biodegradable stents did not become accepted. The authors assumed that through controlled release of growth relevant factors from the biodegradable polymeric scaffolds the potential of this method could be enhanced so that the enhancement especially of cartilage growth would render the reconstructed tracheal segments more stabile [73].

The treatment of subglottic stenoses, especially in children, still is a high challenge in spite of all the progress in surgery. Cotton and Seid in 1980 introduced the anterior cricoid-split [74]. After several modifications of this technique and bearing in mind the contraindications, more than 90% of the children can nowadays be extubated without problems. In spite of the progress, in children undergoing single-step surgical therapy to treat subglottic stenoses it is necessary to use postoperative intubation over several days as an intratracheal splinting. An external splinting by metallic microplates in the surgical tracheal reconstruction was described first time by Zalzal and Deutch in 1991 [75]. Weisberger and Nguyen applied metallic Vitallium miniplates for the external splinting of cartilage transplants in the reconstructive tracheal surgery and 10 of 13 patients (77%) were successfully extubated immediately after surgery [76]. Willner and Modlin introduced resorbable miniplates in the reconstructive tracheal surgery. These resorbable plates were fixed by sutures in the region of the tracheal defect which diminished the stability in comparison to fixation by screws [77]. Following the successful application of resorbable plates and screws made of PLGA in the pediatric craniofacial surgery [78], [79], Long et al. described the external fixation of rib cartilage transplants by PLGA miniplates and screws in the tracheal reconstruction of subglottic stenoses in dogs in 2001. All of the 10 animals operated could be extubated without problems directly postoperatively. In all of these animals there was an adequate widening of the subglottic stenoses over the whole period of observation (up to 90 days postoperatively). Two of the animals developed necroses in the cartilage transplants but in spite of this an endoluminal epithelialization was demonstrated histologically. The 8 other animals showed a complete epithelialization of the transplants [80]. Since the degradation of PLGA in vivo [79] clearly exceeds an observation period of 90 days like in this study, long-term results are missing concerning the resorption of PLGA in tracheal applications and also the

influence of degradation products of PLGA on the mucociliary clearance.

Kojima et al. described the production of tissue engineered tracheal equivalents from cylindrical pieces of cartilage and equipped with an endoluminal epithelium in 2003. Cartilage and epithelial cells were harvested from the septal cartilage of sheep and grown in vitro. After proliferation and cultivation in vitro the cartilage cells were seeded on a PGA matrix. For shaping, the cell polymer scaffold was fixed around a silicon tube and for cultivation under in vivo conditions, the whole construct was implanted under the skin in the back of nude mice. Precultivated epithelial cells were suspended in a hydrogel and injected into the cartilage cylinders. After removal of the stabilizing silicon tubes the tissue engineered constructs were harvested after 4 weeks of implantation. The morphology of the constructs produced by Tissue Engineering was described to be similar to the native sheep trachea. Mature cartilage and the generation of a pseudolayered epithelium were demonstrated histologically. Proteoglycanes and hydroxyproline contents of the constructs were comparable to native cartilage so that the authors assumed that there might be a sufficient stability of such a construct in vivo [81]. It is thought that such a tissue engineered construct in comparison to the earlier applied methods might have the potential to further growth after implantation in vivo which could open new perspectives for the tracheal reconstruction in children. Cartilage was harvested so far from ribs, nasal septum and ears and also from tracheal and joint cartilage. While Kojima et al. assumed that the elastic cartilage from ears might not have the ideal biomechanical properties needed to produce tracheal constructs [81], other authors were less critical in the application of elastic cartilage from ears for the Tissue Engineering of cartilage in tracheal reconstruction [82].

Tracheal resection with the following end-to-end anastomosis is currently the therapeutical “gold standard” in the treatment of tracheal stenoses, when less than 50% of the tracheal length in adults and less than 1/3 of the tracheal length in small children have to be removed [83], [84]. The reconstruction of longer stenoses is a therapeutical challenge not solved at the moment. The tracheal reconstruction of such long segments by transplants necessitates an adequate blood supply to avoid the necrosis of the transplants. Jaquet et al. examined different 3-component grafts in animals to simulate the anatomical structure of the trachea composed of mucosa, cartilage and adventitia. Transplants consisting of cartilage from the ear and from oral mucosa were revascularized through the latero thoracic fascia in rabbits. The epithelialization of 3-component grafts was significantly enhanced through the application of perforated mucosa (40% epithelialization of the constructs after application of perforated mucosa versus 10% epithelialization after application of non perforated mucosa). In all of the 20 operated animals there was a sufficient vascularization, and necroses were not detected in the transplants [85]. The authors assumed that the production of vascularized composite grafts is an option for the reconstruction of longer tracheal stenoses. A successful application of these constructs in animals and clinical studies is missing, however.

A completely different approach for the reconstruction of longer tracheal segments was chosen by other groups who applied aortal autografts for the tracheal reconstruction in pigs [86] and in sheep [87], [88]. In both animals the implants were stabilized postoperatively by silicon stents. Immunosuppression was not applied in either of the animal models. In pigs an epithelialization with metaplastic epithelial cells, newly grown cartilage and non-organized elastic fibres were demonstrated in the implants. In sheep there were initial inflammatory reactions followed by growth of a mucociliary epithelium and the development of new cartilaginous tracheal rings [87]. In 2006 this group published results from the tracheal reconstruction of a longer segment in a human patient applying an aortal autograft. After the resection of a 7 cm long cervical tracheal segment due to a tracheal carcinoma situated directly caudal of the cricoid cartilage and localized clearly intratracheally without regional lymph nodes or distant metastases, there was a tracheal reconstruction applying a segment of the autologous, infrarenal aorta of this 68 year old patient. The excised aortal segment was replaced by a Dacron® prosthesis. A chronical obstructive pulmonary disease (COPD), a peripheral arterial occlusive disease (PAOD) and a myocardial infarction (17 years before the tracheal reconstruction) were known from this patient. The patient was extubated without problems 12 hours postoperatively. There was an endotracheal stabilization applying a silicon stent 3 months postoperatively. An adjuvant irradiation of the whole trachea with 30 Gy was started on the 15th day postoperatively. 4 weeks postoperatively an acute dyspnoe appeared in the patient due to granulation in the region of the proximal anastomosis which was treated with a further stent application proximal to the first stent. Both stents could be removed without problems 3 months later. Afterwards no further granulomatous tissues could be diagnosed endoscopically at the anastomotic sites. Clinically no more states of dispnoea appeared. The patient died due to septical shock in the course of pneumonia in both lungs 6 months post operatively. Since family members did not accept autopsy no further details of the performance of the aorta based tracheal construct could be revealed [89].

Although the aorta based allogenic tracheal constructs did not perform too well in the pig, this approach in 2 animal models and in humans was remarkable both from clinical and from scientific perspectives. From a clinical perspective, the use of aortal segments offers a tubular structure, comparable in diameter to the trachea, which is air and fluid tight, flexible and with high mechanical strength and which is available in the afforded amount. There are problems, however, with the lack of biomechanical stability not avoiding the collapse of air ways and with the missing epithelialization. From a scientific perspective this approach allows the use of decellularized tissues,

even of allogenic ones, as preformed, long distance scaffolds in tracheal reconstruction which enable the ingrowth and differentiation of the patient's own precursor/stem cells assumed to be needed for the regeneration of functional tissues. A lot of efforts in basic science and clinical research have still to be spent until the growth of biomechanically loadable segmental cartilage can be engineered on demand and tissue engineered tracheal constructs will be inhabited by fully functional epithelial cells [90].

2.2.1 Epithelialization of tracheal scaffolds

The first application in humans of an artificial trachea produced according to principles of Regenerative Medicine was published by Omori in 2005. A papillary carcinoma in the thyroid of a 78 years old woman necessitated a hemithyroidectomy together with the resection of the anterior tracheal wall. The tracheal wall defect was reconstructed by a patch based on an Marlex net covered with collagen. 2 months postoperatively, endoscopic analysis revealed the epithelialization of the scaffold. And there was also a sufficient mechanical stability in the scaffold. 2 years after surgery there were still no respiratory complications or insufficiencies. In spite of missing long-term results the authors were convinced that new therapeutical options will be offered for the reconstructive tracheal surgery by Regenerative Medicine [91].
The relatively long period of 2 months needed to epithelialize the patch which was applied in the tracheal reconstruction points to a problem not adequately solved. After application of novel polypropylene collagen scaffolds for the reconstruction of circumscript tracheal defects in dogs, the complete epithelialization of the scaffold could be demonstrated 8 months postoperatively only [92]. A fully functional tracheal epithelium is essential as a physical barrier against the extratracheal milieu, as regulator for the comprehensive metabolic functions of the airways including transport of fluids and ions and for the mucociliary clearance and the patency of the airways [93]. The early development of a complete and functionally adequate epithelialization of tracheal scaffolds is of critical importance for the biofunctionality of implants and constructs produced following the principles of Tissue Engineering. The research on mechanisms of regeneration and differentiation of respiratory epithelial cells in contact with tissue engineered constructs started only recently. Before that, the research concerning the differentiation mechanisms of respiratory epithelial cells was focused on their differentiation in the embryonic phase [94] and on the development and differentiation of epithelial cells from precursor/stem cells [95]. It was shown that basal cells of the human trachea probably are precursors of respiratory epithelial cells [95], [96]. Labelling of cells with specific lectins [97] and applying flow cytometry [98] allowed to recognize basal cells from cells with higher differentiation. Cytokeratines play an important role here as markers of differentiation. The tracheal epithelium is mainly composed of ciliary cells, goblet cells and basal cells [99]. These cells play important roles in the physiology of the airways homeostasis. Ciliary cells regulate the content of fluids and ions and remove body foreign particles through highly coordinated and directed movement of the ciliae [100]. Goblet cells secrete products like mucin which are very important to maintain the barrier function of mucosal layers and for the regulation of cell adhesion [101]. Basal cells are essential for the generation of precursor cells which are fundamental for the regeneration of epithelial damage [95], [96], [102]. Nomoto et al. seeded the scaffold material used by Omori with tracheal epithelial cells of rats in vitro. These epithelial cells expressed in vitro the cytokeratines 14 and 18 as typical intermediate filaments of epithelial cells as well as occludin, a constituent of tight junctions in epithelial cells which is a main component of the barrier against diffusion of soluble substances into the intercellular space. The epithelial cells grown of the scaffold in vitro did not differ immunocytochemically from tracheal epithelial cells in vivo. The scaffolds seeded with epithelial cells in vitro were applied for the reconstruction of cervical tracheal defects of 3 mm length in rats. Over the whole period of observation (30 days) in vivo the artificial trachea was covered with epithelium. However, modifications in the stages of differentiation were observed in the tracheal epithelial cells. Partially, a single or double layered epithelium was found not carrying ciliae whereas other parts displayed prismatic epithelial cells with functional ciliae [103]. In a further development of this technique a thin 3-D collagen matrix (Vitrigel®) was applied for 3-D growth of cells in the scaffold. This 3-D matrix enhanced growth of epithelial cells as well as the invasion of mesenchymal cells. There was a clearly accelerated regeneration of functional epithelial cells carrying ciliae after tracheal reconstruction in rats using Vitrigel® coated scaffolds compared to non-coated scaffolds [104].
The importance of epithelial-mesenchymal interactions for morphogenesis, homeostasis and regeneration of the epithelium are well known from literature since several years [105], [106], [107], [108]. During epithelial regeneration, epithelial precursors arrived from the borders of epithelial damage to proliferate and differentiate there. Mesenchymal cells situated below the epithelium regulate epithelial growth and differentiation through generation of an appropriate biomatrix and through synthesis and release of growth relevant factors [109], [110]. Fibroblasts are also important participants in the interactions between epithelial and mesenchymal cells and strongly influence epithelial regeneration in wound healing. They are able to secrete a variety of growth factors like keratinocyte growth factor, epidermal growth factor (EGF) and hepatocyte growth factor (HGF) [111], [112]. The importance of fibroblasts was shown already for epidermal wound healing [106], oral [113] and corneal epithelial regeneration [114] and in 2006 by Kobayashi et al. also for tracheal epithelial regeneration. The co-cultivation of epithelial cells and tracheal fibroblasts in vitro induced the generation of a layered epithelium containing epithelial cells with ciliae, goblet cells and basal cells. Moreover,

a basal membrane was constituted in vitro between epithelial cells and fibroblasts where the presence of integrin β 4 was demonstrated which is a specific marker of basal membranes and of epithelial mucin secretion [115].
In further studies the authors demonstrated the potential of heterotopic fibroblasts (from dermis, nasal and oral mucosa) for tracheal epithelial regeneration. Regeneration of epithelial cells in contact with different heterotopic fibroblasts showed different characteristics in structure, development of ciliae, secretion of mucins and expression of ion and water channels like e.g. aquaphorines and Na/K ATPase. In contact with nasal fibroblasts, however, no mature and fully functional tracheal epithelium was generated in vitro. Dermal fibroblasts induced the generation of an epidermal like epithelium. Especially the co-cultivation with fibroblasts from the oral mucosa induced the regeneration of a morphologically and functionally regular tracheal epithelium. This was comparable to the regeneration of epithelium in vitro after co-cultivation with tracheal fibroblasts. Fibroblasts from the tracheal and the oral mucosa expressed keratinocyte growth factor, epidermal growth factor and hepatocyte growth factor. Fibroblasts from the oral mucosa enhanced proliferation and migration of epithelial cells in vitro similarly to the tracheal fibroblasts. Since the explantation of oral mucosa is clearly less invasive than the explantation of tracheal mucosa, there seems to be a very promising method available now to develop scaffolds with a functionally adequate epithelium for the tracheal reconstruction [116]. In 2008 the same group used this technique of co-cultivation of epithelial cells and tracheal fibroblasts to produce a tracheal scaffold seeded with cells in vitro and applied the tissue engineered scaffold for the tracheal reconstruction in rats [117]. The authors could demonstrate a fully functional epithelium in vivo. Beside the co-cultivation of tracheal epithelial cells and fibroblasts also the co-cultivation of tracheal epithelial cells and mesenchymal stem cells for the "in vitro" reconstruction of a fully functional tracheal epithelium is described in the literature. The epithelium thus produced showed morphological, histological and functional characteristics of the tracheal mucosa. The authors assumed that the co-cultivation with mesenchymal stem cells could play a main role in Tissue Engineering in future [118].

2.2.2 Vascular supply of tracheal constructs

A problem not adequately solved so far is the vascular supply of scaffolds and of tissue constructs developed from these scaffolds in vivo. A long-term functional epithelium on tracheal constructs necessitates an adequate vascular supply. In contrast to other parenchymal organs the trachea is supplied by a network of small blood vessels which is evidently not easy to generate. Microanastomoses were not successful in animal models [119], [120] and therefore not further persecuted. It is known from the literature that after tracheal reconstruction the capillary network present at the anastomosis proceeded in the direction of the implant only 2 cm at maximum and that this process of revascularization took several months [121]. In tracheal implants which were longer than 3 cm there was a lysis of the epithelium with a consecutive destruction of the basal membrane followed by the development of granulomatous tissues producing a tracheal stenosis. While bioreactors allow the growth of autologous cells [122] and functional tissues and are routinely used for the generation of osteochondral constructs, and tissue engineered heart valves, there are very few studies showing the application of bioreactors for the generation of tracheal scaffolds. Decisive problems hindering the application of tracheal scaffolds in humans are the missing epithelialization and revascularization of the constructs. Tan et al. published in 2006 the concept of a so called "in vivo bioreactor" for the generation of tracheal constructs. They proposed layered scaffolds with a porous catheter within the inner layer of the scaffold for a continuous supply of cells and nutrition media and an outer layer of the construct granting the necessary stability. In contrast to traditional bioreactors where nutrition media mainly flow around the constructs, now a perfusion system was planned within the scaffolds similar to the blood vessel distribution in vivo [123]. This group seeded in a next step a phase segregated multiblock copolymer (DegraPol®) with human tracheal epithelial cells and offered a continuous supply of cells and nutrition media via a porous catheter within the scaffolds. They also examined the influence of vascular endothelial growth factor (VEGF) present in the perfusion medium on the vascularization in the chorioallantois membrane (CAM) assay. The continuous perfusion of the tubular biodegradable scaffolds coincided with an adequate epithelialization of the constructs and an accelerated vascularization in the CAM assay. The authors assumed that the concept of the in vivo bioreactor allows a more physiological process in the reconstruction of tissues and that better initial conditions are granted for the problem so far not solved, the vascularization of tracheal scaffolds [124].

2.3 Regenerative Medicine for reconstruction of pharyngeal defects

The reconstruction of the pharynx by degradable, multifunctional polymeric materials would be a novel therapeutical option in head and neck surgery. The use of implant materials for the reconstruction of pharyngeal defects is currently at the early beginning. Until now there are only data concerning the use of implant materials in the area of the oral mucosa and the palate available. Hallén et al. injected cross-linked hyaluronic acid in rats in the dorsal pharynx wall to treat velopharyngeal insufficiency. In all animals an early inflammatory reaction due to the hyaluronic acid was found. 6 months after injection the hyaluronic acid was still detectable at the original localization of injection and surrounded by connective tissues. Despite lacking of long-term results the authors assumed that the injection of cross-linked hyaluronic acid is appropriate for augmentation of a slight velopharyngeal insufficiency in humans [125]. Ophof et al. implanted skin substrates

after cell seeding with oral keratinocytes in vitro into palatinal wounds in dogs as a model for closure of cleft palate by tissue engineered constructs. In all 6 animals the loss of the epithelium and a distinctive degradation of the skin substrates were detectable. The authors concluded that an adequate integration of these tissue engineered constructs required an early and sufficient revascularization of the scaffolds in vivo [126]. A main focus in Tissue Engineering of oral mucosa is currently the use of novel dermal scaffolds and epithelial cell culture methods including 3-D models. An updated review is given by Moharamzadeh et al. [127].
Despite numerous biomedical applications of tissue engineered constructs in almost all medical fields, there up to now no literature data available regarding the pharyngeal reconstruction with implant materials after tumor resection neither in animal models nor in humans. The availability of multifunctional polymeric implant materials which can be adapted according the anatomical, physiological, biomechanical and surgical requirements [27], [128] facilitate the development of novel therapeutical options also in head and neck surgery. A main scientific topic of the own group is the biocompatibility testing of an elastic degradable AB-copolymer network [28], [29] in vitro and in vivo which seems to be appropriate for the reconstruction of pharyngeal defects due to its physicochemical characteristics.

3 Methods and novel therapeutical options in head and neck surgery

3.1 Primary cell cultures of the upper aerodigestive tract

The use of cell culture is an essential tool in nearly all biological and medical research laboratories. The biocompatibility testing should be conducted with cultures of site-specific cells depending on the biomedical application to assess the specific interaction between the biomaterial and site-specific different cells [129]. Thus, the biocompatibility testing of a polymeric materials which seems to be appropriate for the reconstruction of pharyngeal defects should be conducted with primary cell cultures of the pharynx. The knowledge about the interactions between the implant materials and cells/tissues is a basic requirement for an ideal adaptation of a polymeric material according to the specific needs of the upper aerodigestive tract. In own studies primary cell cultures of the oral cavity, the pharynx, and the esophagus were established and biochemically characterized. Immuncytological investigations showed different relative amounts of epithelial, fibroblastic and smooth muscle cells depending on the anatomical site of explantation [130]. Relatively little is known about the mechanisms of regular and delayed wound healing of the pharyngeal epithelium. Therefore, a comprehensive characterization of primary cell cultures of the pharynx was a first step for the development and establishment of novel therapeutical options [130], [131].

3.2 Assessment and regulation of matrix metalloproteases and wound healing

The cell adhesion, -migration, -proliferation, angiogenesis, degradation of extracellular matrix and remodeling of granulation tissue are decisive steps in wound healing [132]. The amount and organization of the extracellular matrix in normal wounds is determined by a dynamic balance between overall matrix synthesis, deposition and degradation. A strictly controlled degradation of the extracellular matrix is an important process for the regular wound healing. An imbalance between degration and synthesis of the matrix during wound healing would cause a delayed wound healing with fistulae and ulcerations in case of outbalanced degradation of the extracellular matrix or hypertrophic scars and keloids in case of outbalanced synthesis of the extracellular matrix [133].
Matrix metalloproteases (MMPs) are a class of structurally related, zinc-dependent endopeptidases that are collectively responsible for the degradation of extracellular matrix proteins. MMPs have an important function in wound healing [134], [135]. Under regular conditions in vivo, the expression and activation of MMPs is strictly controlled. The activity of MMPs is regulated at the level of transcription and zymogen activation and can be inhibited by specific inhibitors: the tissue inhibitors of metalloproteases, TIMPs. Recently, 4 different TIMPs (TIMP 1-4) were identified and cloned [136]. In the literature different MMP- und TIMP-levels were reported in regular and delayed wound healing [137], [138]. The delicate balance between the activity of MMPs and TIMPs plays a key role in building a functional extracellular matrix. Up to know little is known about the mechanisms of wound healing and MMP expression of cells of the upper ADT in vitro and in vivo [139], [140], [141].
A comprehensive characterization of the MMP- and TIMP expression of cells of the upper ADT is a basic requirement to develop and establish novel therapeutical options in head and neck surgery in case of delayed wound healing after surgical treatment. A main focus of the own biocompatibility testing was the analysis of the MMP- and TIMP expression of primary cell cultures of the upper ADT after cell seeding on different modifications of the polymeric implant material to gain the knowledge for an optimal adaptation of these materials to the specific requirements of the upper ADT.
Among the primary cell cultures investigated, cells of the pharynx were seeded on the surface of a multifunctional copolymer as well as on the surface of commercially available polystyrene cell culture dishes as control. On both surfaces cells became adherent, proliferated and reached confluency. No statistically significant differences of the mean cell numbers were found on Day 1, 3, 6, 9 and 12 of cell growth after cell seeding [131]. The highest MMP-1-, MMP-2- and TIMP-levels were found on Day 1 of cells grown on both surfaces. There were decreasing

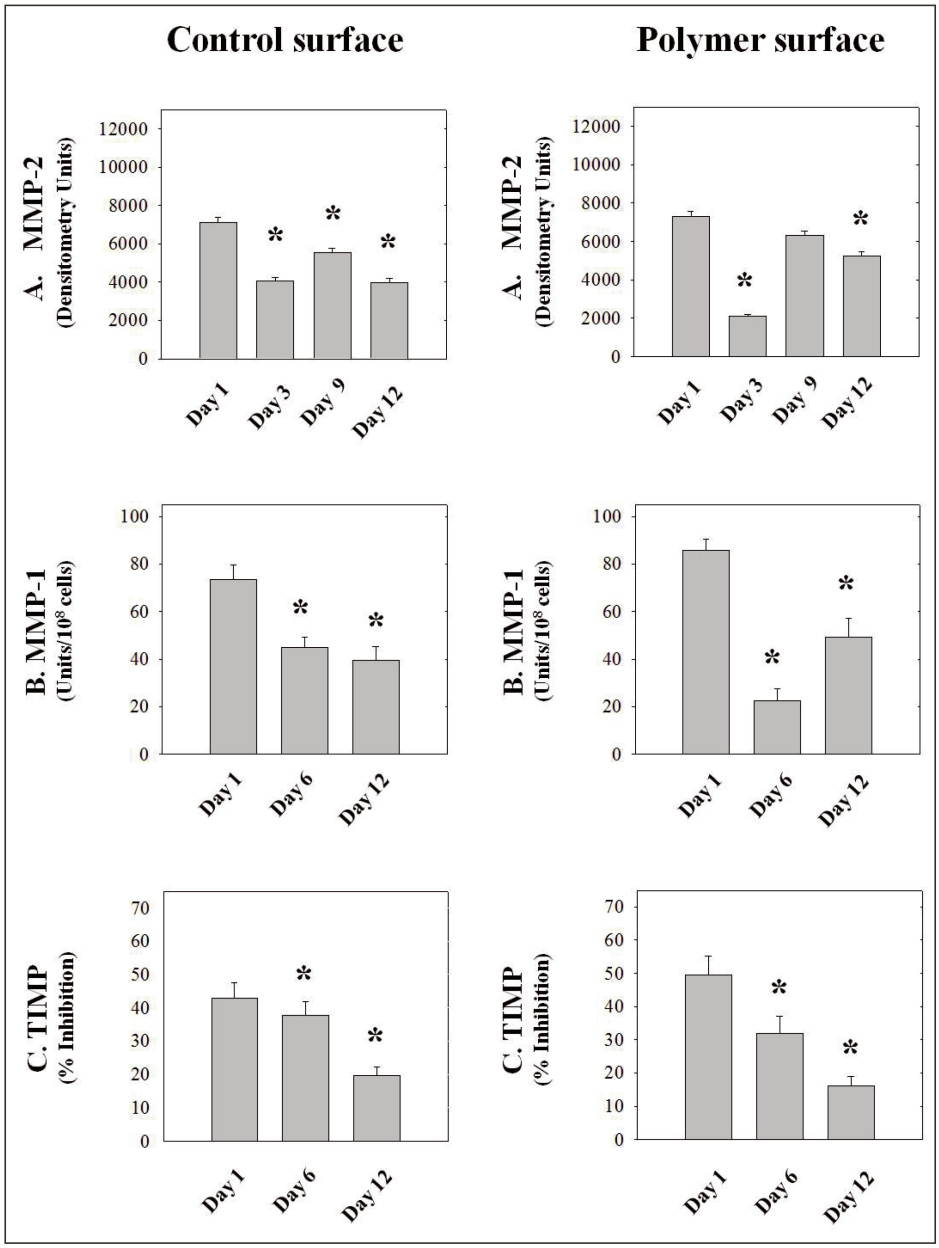

Figure 2: Kinetics of appearance and activity levels of MMP-2, MMP-1 and TIMPs of primary cell cultures of the pharynx grown on polystyrene (control surface) versus multiblock copolymer surface

Scanning densitometry units of the gelatinolytic activity of MMP-2 are shown in A. MMP-1 activity is represented as units per 108 cells in B. The TIMP activity was calculated as percent inhibition of MMPs (C). MMP-2 levels were determined on Days 1, 3, 9 and 12 of cell growth. MMP-1 and TIMP levels were analysed on Days 1, 6 and 12 of cell growth. Statistical analysis was performed to determine differences in MMP-2, MMP-1 and TIMP levels between Day 1 and the subsequent days of cell growth. Statistically significant differences ($p \leq 0.01$) are indicated by a star. Data shown are averages from three separate experiments with 9 cell culture each (values are means ± s.d.).

levels during the following time of the investigation (Figure 2). No statistically significant differences of the MMP- and TIMP-expressions were detectable between the polymer and the control surfaces. The kinetic of MMP-2 expression were analysed on the protein level and by RT-PCR on the mRNA level (Figure 3) [131]. Based on the current results the adhesion, proliferation, and differentiation of the primary cell cultures of the pharynx was not influenced by the multifunctional copolymer.

3.3 Influence of implant topography

The integration of a material in the surrounding tissues is a basic requirement for a successful clinical application of an implant material in vivo. The surface characteristics of materials including their surface topography and chemical composition are of very high importance for the interaction between the material and cells and tissues [142], [143], [144], [145]. Until know some cellular processes are known which could be useful to assess the cellular behavior on implant materials. Most of this

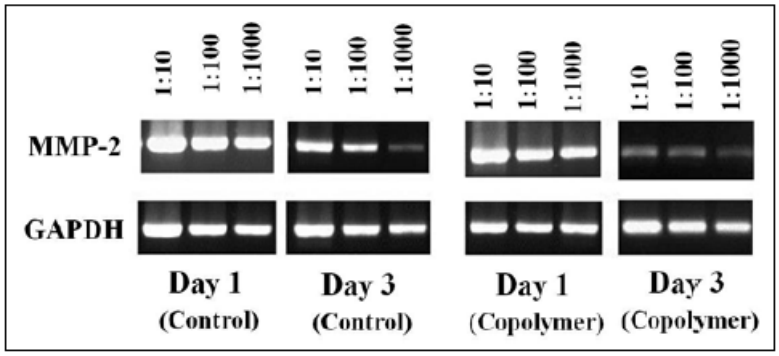

Figure 3: mRNA levels of MMP-2 of primary cell cultures of the pharynx grown on polystyrene (control) versus multiblock copolymer surface

MMP-2 mRNA levels of primary cells of the pharynx on Day 1 and 3 grown on polystyrene (control) are shown on the left side. The kinetics of MMP-2 mRNA levels on Day 1 and 3 of cells grown on copolymer are presented on the right side. Primers used for RT-PCR were rat MMP-2 and rat GAPDH.

Abbreviations: RT-PCR = Reverse Transkriptase Polymerase Chain Reaction

knowledge is based on cell culture investigations and it is unknown if these mechanisms are also found in vivo [146], [147]. A fundamental requirement for a successful application of degradable implant materials for the pharyngeal reconstruction in vivo is a saliva-tight integration of the material in surrounding tissues. Furthermore, an adequate chemical stability of the implant material is needed to avoid salivary fistulae with destruction of neighbouring soft tissue. The development of long-term degradable polymeric scaffolds for pharyngeal reconstruction has to guarantee an adequate biocompatibility and biofunctionality as well as growth of a functional tissue formation considering the specific physiological and mechanical requirements of the upper ADT. Important progress in biomaterial research of the last years was made in the improvement of cell adhesion and -proliferation by the optimization of scaffold design with respect to specific requirements of the different implantation sites in vivo [148]. Main aspects of the the research work were focused on the influence of different macroscopical and microscopical design parameters on the local differentiation of variable cells. Other aspects dealt with the controlled release of growth factors [149], [150]. Until now relatively little is known about the influence of different surface topographies of polymeric implant materials on the gene expression and synthesis of enzymes that are directly involved in extracellular matrix remodeling [151], [152].

Our own results demonstrated the importance of the surface structure of polymeric implant materials on the cellular behavior depending on surface roughness (smooth versus rough surfaces). The cell adhesion, -proliferation, as well as the kinetics of secretion and activity of MMP-1, MMP-2- and TIMPs differed significantly depending on the type of cells and on the surface structure of the copolymer. Significantly greater average total cell numbers of oral and pharyngeal primary cells were found after cell seeding on the rough surface compared to the smooth polymer surface. Esophageal cells showed the highest cell numbers on the control polystyrene surface. Oral and pharyngeal cells revealed similar kinetics of appearance and activity of MMP-1, MMP-2 and TIMPs with highest values on Day 1, followed by a decrease of the activity levels on the rough polymer and the control surface. Oral and pharyngeal cells seeded on the smooth polymer surface displayed an opposite pattern with the lowest activity of MMP-1, MMP-2 and TIMPs on Day 1 and highest values on Day 12. Esophageal primary cell cultures showed a comparable kinetic pattern of appearance and activities on all three different surfaces (smooth and rough polymer surface, control surface) with the lowest MMP-1-, MMP-2- and TIMP expression on Day 1 and highest values on Day 12 [153].

The presence or absence of the extracellular matrix or components of it govern the proliferation, differentiation and biochemical activities of the different primary cell cultures of the upper ADT. These results were confirmed by data from the literature which also showed the influence of the surface topography on the gene expression and synthesis of the enzymes directly involved in extracellular matrix remodeling [154], [155], [156].

The results of these experiments suggest a specific influence of surface topography on the behavior of cells in contact with implant materials. The knowledge of the exact mechanisms of the cell-biomaterial-interactions are a basic requirement for the development of an “ideal” implant material to establish cell- and tissue-optimized novel therapeutical options in head and neck surgery based on polymeric implant materials.

3.4 Application of new implant materials in animal models

The use of degradable implant materials in the area of the upper ADT makes high demands on the chemical, enzymatical, bacterialand mechanical stability of a material. A premature degradation of the implant material would cause extensive salivary fistulae with high mortality potentially culminating in carotid artery rupture. Because of the chemical conditions in the upper ADT with changing pH-values, enzymatical, bacterial and particular mechanical load during deglutition and digestion the reconstruc-

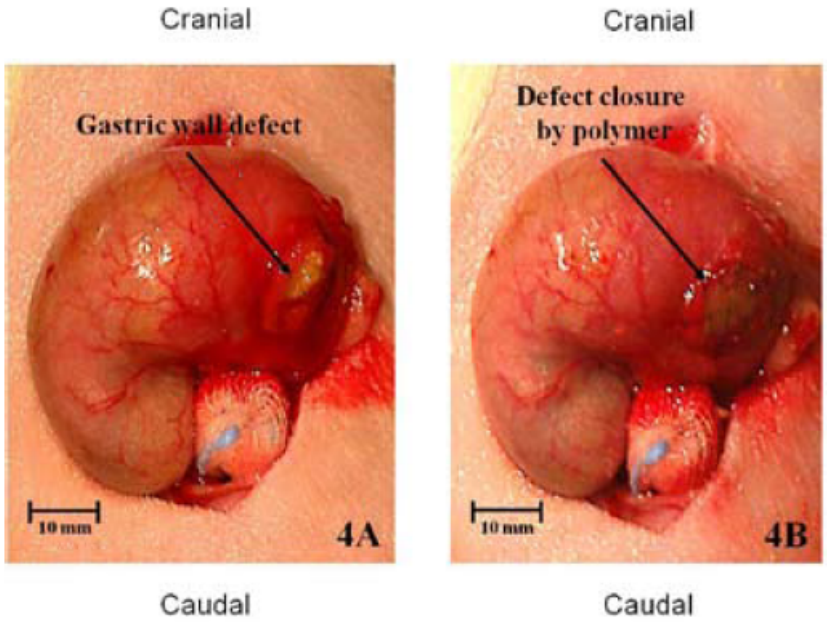

Figure 4: The gastric wall defect is shown in Figure 4A. The closure of the defect by a multiblock copolymer in the implantation group is presented in Figure 4B.

A standardized gastrotomy with a diameter of 10 mm was performed at the ventral side of the stomach between the smaller and the greater curvatures. The defect of the gastric wall is marked by an arrow (Figure 4A). In the implantation group the defect was closed with a copolymer patch (diameter 10 mm; thickness 200 µm). The defect closure by a copolymer patch is marked by an arrow (Figure 4B). The copolymer patch was sutured into the gastric wall defect with a monofil, non-resorbable 8/0 thread in a non-interrupted sero-muscular technique.

tion of the upper ADT by a degradable implant material requires adequate chemical, enzymatical, bacterialand mechanical stabilities of the scaffold material. Until now these comprehensive needs can be tested only in animal models. In our own group an animal model was established creating a standardized complete defect of the gastric wall in rats which was closed by an elastic long-term degradable polymeric implant (Figure 4). The stomach was used as a "worst case" application site to test the stability of the implant material under extreme chemical, enzymatical, bacterial, and mechanical load. In this model the mortality of the gastric breakdown of sutures and fistulae implying local or generalized peritonitis are comparable to the mortality of insufficiencies and salivary fistulae of the pharynx. The implantation group included 42 animals. A primary wound closure of the gastric wall defect without biomaterial implantation was conducted in the control group (n=21). Furthermore, a so called baseline group which inclueded animals kept under the same conditions without any surgical procedure was investigated (n=21). The implantation periods or times of observation were 1 week, 4 weeks and 6 months [157], [158].

Fundamental parameters investigated in this animal model were a tight closure between the polymer and surrounding tissues, the chemical and mechanical stability of the implant material, the integration of the polymer in the surrounding tissue as well as the question of tissue regeneration after reconstruction of the defect with the polymeric implant material. Gastrointestinal complications like fistulae, perforation or peritonitis did not occur in any of the animals. A liquid- and gas-tight anastomosis between the polymer and the adjacent stomach wall existed in all animals of the implantation group (Figure 5) [157]. To test the impermeability between the implant material and adjacent gastric wall the intragastric pressure was measured after maximal dilatation of the stomach by air insufflation (Figure 6) [157]. Neither in the implantation nor in the control group a delayed wound healing was observed macroscopically or microscopically after 1 week, 4 weeks and 6 months of implantation time respectively after primary wound closure. After 1 week a beginning regeneration of the gastric wall was detected starting from the border area of the gastric wall defect. After 4 weeks and 6 months a regular multi-layered stomach tissue as known from histology was found in the former defect zone of the gastric wall (Figure 7). In the control group the defect was replaced by scar tissue [158]. The analysis of the mechanisms of the integration of the implant material in the adjacent tissues as well as the mechanisms of tissue regeneration are topics of currently ongoing examinations. The importance of the biocompatibility and biofunctionality of the implant material on the tissue regeneration were further enlighted by completely different results in animal experiments with poly-L-lactid as the current "gold standard" of degradable implant materials (unpublished results). After an attempted reconstruction of the gastric wall with poly-L-lactid the same animal model, this examination had to be terminated after 12 animals due to perforation of the gastric wall and extensive peritonitis in 9 of the 12 of the animals (75%) (Figure 8).

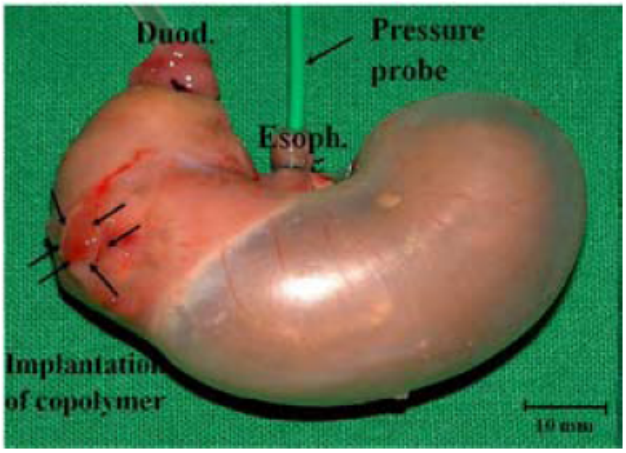

Figure 5: Aspect of the explanted stomach after 1 week of copolymer implantation

The polymer implantation site is marked by arrows. A flexible tube for air insufflation was inserted in the duodenum. The pressure was measured by a probe in the resected esophagus. The pressure probe is marked by an arrow. A special anatomical feature of the rat stomach becomes overt: the stringent separation between the glandular part of the stomach where the copolymer was implanted (marked by arrows) and the non-glandular part. The influence of this special anatomical feature on the biofunctionality of the polymeric material is unknown so far and needs to be investigated in another animal model.

Abbreviations: Duod. = Duodenum; Esoph. = Esophagus

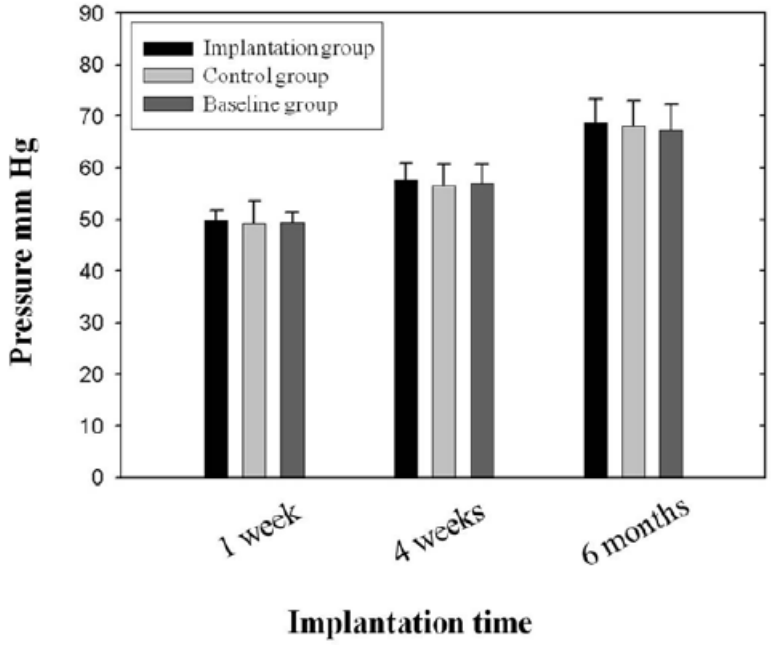

Figure 6: Graphical presentation of the measurement of the intragastric pressure (mm Hg) after maximal dilatation of the stomach by air insufflation

Measurement of the intragastric pressure (mm Hg) after maximal dilatation by air insufflation in the implantion, the control and the baseline group after 1 week, 4 weeks and 6 months

Furthermore, the systemical influence of the copolymer was investigated. It is well known from literature that the peritoneum is a very sensitive compartment for inflammatory reactions of the organism dependent on the biocompatibility of implant materials [159]. Incompatibilities of implant materials and/or their too early degradation are expected to cause local inflammatory reactions originating acute-phase-reactions concomitant with the induction of gene expression of acute-phase-proteins. The concentrations of the acute phase proteins α1-Acid Glycopotein and Haptoglobin, however, did not show statistically significant differences between the multiblock copolymer and the control group [160].

In the experiments performed until now the chemically, hydrolytically and enzymatically stability as well as the biomechanical functionality of the polymeric implant material were shown under the extreme conditions of the stomach. The postoperative increase in weight of the animals [157], the impermeability between the implant material and adjacent tissues of the gastric wall [157], the concentrations of the acute-phase-proteins α1-Acid Glycopotein and Haptoglobin [160] as well as the lack of

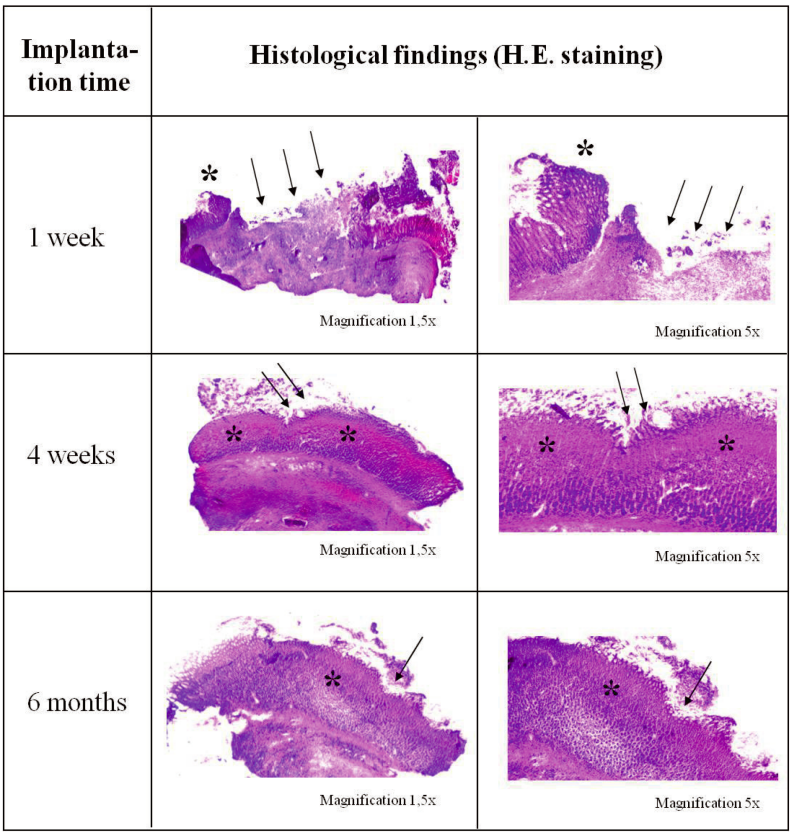

Figure 7: Histological findings after 1 week, 4 weeks and 6 months of implantation time
The defect of the gastric wall (marked by arrows) is characterized by the lack of the stomach epithelium in both magnifications (1.5x, 5x) after 1 week of implantation time. The marginal area next to the defect zone showed a regular stomach epithelium marked by stars. After 1 week of implantation time a beginning tissue regeneration was detectable from the marginal area next to the defect zone. The polymeric material used for defect closure was removed due to the xylene and ethanol treatment and cutting of paraffin sections and was not detactable on most of the histological sections. After 4 weeks and 6 months of implantation time in all animals a histological regular-layered gastric wall was detected in the former defect zone (marked by arrows). The marginal area of the former defect is marked by stars.
Abbreviations: H.E. staining = Hematoxylin Eosin staining

gastrointestinal complications suggest that the wound healing was not negatively influenced by the degradable multiblock copolymer during the time investigated. On the contrary, a support of tissue regeneration by the implant material was detected. The mechanisms of biomaterial-tissue-integration, of tissue regeneration, of tissue remodeling as well as the mechanisms of polymer degradation have to be analysed in future experiments.

The results available so far regarding the tissue compatibility allow to regard the copolymer network as a very promising implant material for the development of novel therapeutical options in head and neck surgery based on degradable biomaterials. In a next step the copolymer will be used for the reconstruction of the upper ADT at first in a large animal model. If there is a positive evaluation in future and also in clinical studies, applications of this polymer become conceivable for the reconstruction of the pharynx in humans.

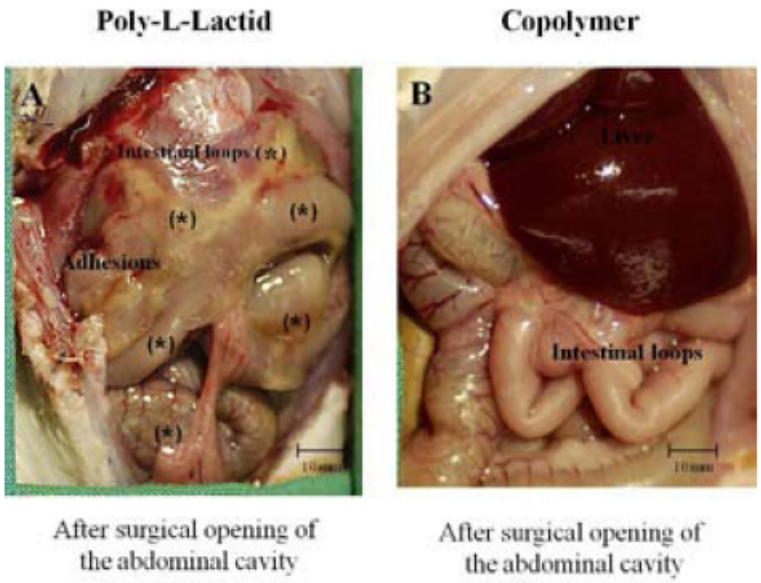

Figure 8: Macroscopical findings in the abdominal cavity after application of Poly-L-Lactid (A) or Copolymer (B) in the gastric wall of rats

After 1 week of implantation time after application Poly-L-Lactid (A) in the gastric wall distinctive adhesions of the whole abdominal cavity were found. The animal experiments with Poly-L-Lactid had to be terminated after 12 animals because of perforation of the gastric wall and serious pertonitis in 75% of the animals. In contrast to these findings a mirroring peritoneum was detected in all animals of the implantation group (n=42) after reconstruction of the gastric wall by the Copolymer without adhesions of the intestinal loops. Gastrointestinal complications like perforation or peritonitis did not occur in any one of the animals of the implantation group.

4 Vascularization of tissue engineered constructs

The vitality and functionalitiy of tissue engineered constructs depends on an adequate blood supply with oxygen and nutrients as well as on the removal of metabolites. Most of the tissues/organs successfully tissue engineered until now are relatively thin and/or avascular like cartilage, skin or urinary bladder. Therefore, wound healing driven angiogenesis in recipients is thought to be sufficient to supply the tissue engineered constructs with oxygen and nutrients in many cases. It was suggested that the supply of blood and nutrient of the scaffolds applied for pharyngeal reconstruction could be sufficient because the used implant materials are relatively thin (<100 µm). In any case, the applied scaffolds should support angiogenesis. The investigation of the influence of polymeric implant materials on the angiogenesis is therefore an important aspect of biocompatibility testing. In our investigations we showed that bovine capillary endothelial cells (EC) of the adrenal cortex [161] became adherent on the copolymer surface and developed confluent cell layers [162]. Also, in the chorioallantois membrane (CAM) assay no negative influence of the copolymer samples on the vascularisation was detectable [162], [163]. A controlled release of angiogenic factors from vesicles on the polymer surface according to the principles of Drug Delivery to support angiogenesis is a scientic topic of currently ongoing investigations.

At present an adequate vascularisation of the cellularly colonized scaffolds in vivo is one of the most critical points for Tissue Engineering of complex and metabolic challenging organs like heart or liver. In case of parenchymal organs the tissue engineered microcirculation has to be connected to the recipients circulation. The currently available techniques for the vascularisation of tissue engineered constructs can be classified in “in vitro” and “in vivo” methods. In the last years considerable progress was made to solve the problems of building microcirculatory networks for comprehensive 3-D constructs. Kunz-Schughart et al. developed a 3-D cell culture system with co-cultivation of human skin fibroblasts and endothelial cells of the umbilical cord. They found a support of migration, vitality and development of tubular structures of the endothelial cells by fibroblasts. Based on such models knowledge about the integration of capillary structures in engineered tissues can be gained [164]. Au et al. suggested as approach for the vascularisation of tissue engineered scaffolds the co-cultivation of constructs with blood vessel cells like endothelial and perivascular cells. The authors demonstrated that the co-implantation of the scaffolds with site-specific cells and with endothelial and perivascular cells led to the development of vascular structures in vivo connecting the scaffolds and recipient’s circulation. The stability and adequate functionality of these vascular structures remained now for more than 1 year. Based on these results the authors assumed that this technique of co-implantation is a promising approach for the vascularisation of tissue engineered constructs [165].

On the other side there are still numerous unsolved problems like the connection of scaffolds to the recipient’s vascularisation, the maintenance or increase of

vascular density with an increase of tissue or organ mass or activity, the maturation of functionally inadequate vessels as well as the unwanted regression of vascular structures. One of the answers to these problems might be gained in future through a comprehensive knowledge about the regulation of the heterogeneous endothelial cells in different organs. Furthermore, an extensive knowledge about the mechanisms of the molecular processes of cellular interactions between endothelial cells, pericytes and smooth muscle cells and between blood vessels and parenchymal cells are needed. Beyond that, the mechanical characteristics of blood vessels like permeability, elasticity and compressibility have to be analysed and the design non-thrombogenic surfaces of implant materials have to be devised. A review about the current knowledge of microcirculation engineering as a basic requirement for a successful Tissue Engineering of parenchymal organs is given by Lokmic et al. [166].

5 Application of stem cells in Regenerative Medicine

Stem cells have the capacity for self-renewal and capability of differentiation to various cell lineages. Thus, they represent an important building block for Regenerative Medicine and Tisue Engineering. These cells can be broadly classified into embryonic stem cells and non-embryonic or adult stem cells. Embryonic stem cells are called pluripotent and can differentiate in all cell types of the 3 embryonic germ layers. On the other hand, the adult stem cells are multipotent and the differentiation of these cells is terminated to only 1 of the germ layers. Embryonic stem cells have a great potential but their use is limited by several ethical and scientific considerations which were the basis for the German law. Limited factors for the use of embryonic and adult stem cells next to ethical considerations [167], [168] are problems associated with extensive in vitro cell expansion [169], problems with in vitro cultivation on implant materials [170], [171], cell apoptosis following implantation [172], as well as vascularisation [173] and fincancial problems of stem cell technology [174].

Stem cells were already studied by Becker et al. 1963 who injected bone marrow cells into irradiated mice and noticed that nodules developed in the spleens of the mice in proportion to the number of bone marrow cells injected [175]. They concluded that each nodule arose from a single marrow cell. Later on, they found evidence that these cells were capable of infinite self-renewal, one of the central characteristics of stem cells.

Stem cells have been used successfully in experimental and clinical studies for bone, cartilage, spinal cord, cardiac and bladder regeneration. A current review about the application of stem cells in the field of Regenerative Medicine is given by Bajada et al. [12].

2001 Vacanti et al. reported the successful Tissue Engineering of the distal phalanx and the replacement of this bone in a 36 year old patient who suffered partial avulsion of the thumb [176]. However, only 25% of the normal strength were obtained. Quarto et al. reported on the use of autologous culture-expanded bone marrow stromal cells (BMSCs) combined with porous hydroxyapatite for the reconstruction of critical sized defects (bone segemental defects 4–7 cm long) of tibia, ulna and humerus. The results were encouraging, with good graft integration and return to functionality [177]. Hibi et al. published in 2006 the use of Tissue Engineering to augment bone formation in humans in combination with vertical distraction osteogenesis (DO) by an osteocutaneous fibulartransplant for the reconstruction of the mandible after irradiation. DO is a method for elongation of the bone which is used among others in the surgical reconstruction of facial skull to bridge bony defects of different genesis. To promote 3-D bone formation and shorten the consolidation period, the authors applied tissue-engineered osteogenic material ("injectable bone") in a patient who was treated with vertical DO and an osteocutaneous fibular flap to reconstruct the mandible. The material, which comprised autologous mesenchymal stem cells was culture-expanded and then induced to be osteogenic in character. Platelet-rich plasma (PRP) was activated with thrombin and calcium chloride and infiltrated into the distracted tissue at the end of distraction and injected into a space created labially with a titanium mesh at implant placement. The reconstructed mandible was expanded from 10 mm to 25 mm in height despite a lacerated and opened labial periosteum in the distracted area. The authors assumed that DO assisted by Tissue Engineering could be the therapy of choice in future for the surgical reonstruction of bony defects [178]. Furthermore, the authors used this technique of tissue engineered osteogenic material („injectable bone") successfully as well for the osteoplastic reconstruction in cleft palate in a 9 year old girl [179].

While stem cells are successfully in clinical use for the regeneration of articular cartilage since several years [180], the complete reconstruction of the auricle by Tissue Engineering is still a great challenge in head and neck surgery. The reasons are complex and especially related to the unsolved problems of scaffold design and of the differentiation induction of stem cells to produce elastic ear cartilage [181]. There are numerous other less attended fields of research in head and neck surgery needing stem cell technology, e.g. the mucosal reconstruction in the upper ADT. A first approach is the development of ciliated epithelium by co-cultivation of stem cells with site-specific cells [182].

While these technologies are already in use for the reconstruction of the mucosa of the urinary tract [183] and the cornea [184] and for teeth regeneration [185], the development of mucosal reconstruction in head and neck surgery except for the salivary gland tissue [186], [187], [188] is still at the relative beginning.

6 Conclusion

The quality of an implant material is exclusively shown in a successful clinical use. The profil of demands is therefore determined by the conditions in vivo. The chemical, enzymatical, bacterial and mechanical conditions of the upper ADT make high demands on an implant material for the mucosal reconstruction in this area. In reconstructive surgery of the trachea, none of the different implant materials investigated by versatile methodical approaches was successfully introduced in the clinical use. For the reonstruction of pharyngeal defects based on the principles of Regenerative Medicine until now there exist neither animal models nor a clinical application in humans. Based on the progress in polymer chemistry multifunctional implant materials are available nowadays which can selectively initiate biological processes in a physiological environment and/or change their physicochemical characteristics in reaction to external stimuli. The availability of such multifunctional implant materials and the progress in Tissue Engineering resulted in the establishment of novel therapeutical options in different medical fields. Applying stem cell technology further progress is expected for the reconstruction of different tissues based on the principles of Tissue Engineering. To benefit from the potential of such technologies for the development and the establishment of novel therapeutical options in head and neck surgery, clinicians have to be involved in these interdisciplinary scientific projects of Regenerative Medicine.

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