Final Draft
of the original manuscript

Rickert, D.; Steinhart, H.; Lendlein, A.:
**Functional requirements for polymeric implant materials in head and neck surgery.**

First published online by IOS: 07.09.2020

[https://dx.doi.org/10.3233/CH-209212](https://dx.doi.org/10.3233/CH-209212)
Functional requirements for polymeric implant materials in head and neck surgery

Short title: Biomaterials in head and neck surgery

Dorothee Rickert¹,²*, Helmut Steinhart², Andreas Lendlein¹,³

¹ Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies, Helmholtz-Zentrum Geesthacht, Kantstraße 55, 14513 Teltow, Germany
² ENT-clinic, Department of Head and Neck Surgery, Marienhospital Stuttgart, Böheimstraße 37, 70199 Stuttgart, Germany
³ Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Str. 24/25, 14476 Potsdam, Germany

* To whom correspondence should be addressed:
Dr. Dorothee Rickert, ENT-clinic, Department of Head and Neck Surgery, Marienhospital Stuttgart, Böheimstraße 37, 70199 Stuttgart, Germany
Phone: +49 711 64897112
E-mail: dorothee.rickert@vinzenz.de
Abstract

Background: The pharyngeal reconstruction is a challenging aspect after pharyngeal tumor resection. The pharyngeal passage has to be restored to enable oral alimentation and speech rehabilitation. Several techniques like local transposition of skin, mucosa and/or muscle, regional flaps and free vascularized flaps have been developed to reconstruct pharyngeal defects following surgery, in order to restore function and aesthetics. The reconstruction of the pharynx by degradable, multifunctional polymeric materials would be a novel therapeutical option in head and neck surgery.

Materials and Methods: Samples of an ethylene-oxide sterilized polymer (diameter 10 mm, 200 µm thick) were implanted for the reconstruction of a standardized defect of the gastric wall in rats in a prospective study. The stomach is a model for a “worst case” application site to test the stability of the implant material under extreme chemical, enzymatical, bacterial, and mechanical load.

Results: Fundamental parameters investigated in this animal model were a local tight closure between the polymer and surrounding tissues, histological findings of tissue regeneration and systemic responses to inflammation. A tight anastomosis between the polymer and the adjacent stomach wall was found in all animals after polymer implantation (n=42). Histologically, a regeneration with glandular epithelium was found in the polymer group. No differences in the systemic responses to inflammation were found between the polymer group (n=42) and the control group (n=21) with primary wound closure of the defect of the gastric wall.

Conclusions: A sufficient stability of the polymeric material is a requirement for the pharyngeal reconstruction with implant materials.
Keywords

Oncological head and neck surgery; pharyngocutaneous fistulae; multifunctional polymeric materials; reconstruction of pharyngeal defects; animal model

Introduction

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. 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 therapeutic interventions. Surgery with or without adjuvant therapy and radiotherapy with or without chemotherapy have traditionally represented the possible treatment options for oropharyngeal cancer. A pharyngocutaneous fistula is a common and difficult-to-manage complication after head and neck reconstruction. It can lead to serious complications such as flap failure, carotid artery rupture, and pharyngeal stricture, and may require additional surgery [1]. Pharyngocutaneous fistulae result in prolonged hospitalization with increased medical costs, delay of adjuvant postoperative therapy and potentially life-threatening complications such as carotid rupture [2]. Surgeons have developed various reconstructive techniques to minimize the rate of pharyngocutaneous fistula and optimize functional outcome after salvage laryngectomy or laryngopharyngectomy. Vascularized tissue augmentation reduces the overall fistula rate and fistula requiring reoperation but vascularized tissue augmentation with muscle may impair speech and swallowing outcomes [3]. The limited results in speech and swallowing function cause a serious reduction of quality of life for the patients, high costs in health systems as well as high costs in social
security systems because of limited employability in younger patients. Incidence of oropharyngeal carcinoma (OPSCC) is increasing significantly worldwide. Human papillomavirus (HPV) infection is established as the main explanation [4]. High percentage of HPV-induced tumors of the upper aerodigestive tracts were diagnosed in an advanced stage. The socioeconomic change requires a different assessment of the oncological therapy in head and neck tumors concerning functional aspects. Next to tumor freedom functional speech and swallowing outcomes gain more and more weight [5-7].

Regeneration as a therapeutic principle and regenerative medicine in general are promising new strategies to add novel therapeutical options to our current treatment strategies in Otorhinolaryngology. Reconstructive surgery can replace the functions of damaged tissues. In contrast, the aim of regenerative therapies is to restore structure and function following tissue resection. Almost all fields in Otorhinolaryngology can be the aim of regenerative therapeutical options. Regenerative methods are increasingly used in clinical studies, but are rarely used in the clinical routine because of different reasons [8]. Main causes are conditioned by obstacles in the preclinical, clinical, commercial and regulatory field [9]. In the clinical field risk management, ethical concerns and a not suitable study design are among others some main points to impede or prevent the step from bench to bedside for regenerative therapies.

The role of biomaterials has become more important in the last 30 years in otologic and nasológica. The indications for implants are generally similar to those of autografts.

To focus on the material side in order to improve the functional speech and swallowing outcomes after surgical oropharyngeal reconstruction would be a different approach. Pharyngeal reconstruction is a challenging aspect of reconstruction after resections for head and neck cancer. The goal of reconstruction is to restore the continuity of the
pharyngeal passage to enable oral alimentation and rehabilitation of speech wherever possible. Several techniques have been developed to reconstruct oral and pharyngeal defects following surgery, in order to restore function and aesthetics. These are primary closure, skin grafts, local transposition of skin, mucosa and/or muscle, regional flaps and free vascularized flaps [10,11]. Advantages and limitations of the different surgical techniques are shown in the literature [12].

The reconstruction of the pharynx by degradable, multifunctional polymeric materials would be a novel therapeutic option in head and neck surgery. Until now there are only data concerning the use of implant materials in the area of the oral mucosa and the palate available [13,14].

A main focus in tissue engineering of oral mucosa is currently the use of novel dermal scaffolds and epithelial cell culture methods including three-dimensional (3D) models. A review is given by Moharamzadeh et al. [15].

3D printing has numerous applications and has gained much interest in the medical world. The constantly improving quality of 3D printing applications has contributed to their increased use on patients. The reports on 3D printing outcomes concern multiple surgical domains. Orthopedics has the largest share, followed by maxillofacial surgery and cranial surgery [16].

3D printing with biomaterials in craniofacial and dental tissue engineering has already been an important kind of therapeutic method in craniofacial and dental field, such as trauma, skeletal disease, wound surgery and periodontal disease. A review of these techniques in craniofacial and dental tissue engineering is given by Liao et al. [17].

Medical 3D printing, the fabrication of handheld models from medical images, has the potential to become an integral part of otolaryngology-head and neck surgery with broad impact across its subspecialties. Widespread implementation of 3D printing in Otorhinolaryngology and Head and Neck Surgery is still at its infancy. Nonetheless, it
is increasingly being utilized across all subspecialties from preoperative planning to design and fabrication of patient-specific implants and surgical guides. An emerging application considered highly valuable is its use as a teaching tool for medical education and surgical training. A review of the basic principles of this technology and a comprehensive summary of reported clinical applications in the field is given by Hong et al. [18].

Major limitations of utilizing 3D printing technology include time and cost, which may be offset by decreased operation times and collaboration between departments to diffuse in-house printing costs. The current state of the literature shows promising results, but has not yet been validated by large studies or randomized controlled trials [19].

Biomedical applications of tissue engineered constructs for pharyngeal reconstruction is in contrast to a lot of other medical fields in head and neck surgery at the very beginning [20-22]. Up to now, there are no data in the literature available regarding pharyngeal reconstruction exclusively with implant material after tumor resection neither in animal models nor in humans. A combination of artificial biological material – acellular dermal matrix (Alloderm, ADM) – combined with myocutaneous flaps is described in the literature for hypopharyngeal reconstruction [23]. The availability of multifunctional polymeric implant materials, which can be adapted according to the anatomical, physiological, biomechanical and surgical requirements [24-29] facilitate the development of novel therapeutic options also in head and neck surgery.

In addition to cell compatibility [30], sterilizability is a further necessary property of biomaterials. Polymer-based and especially hydrolytically degradable biomaterials in general have a considerably lower thermal and chemical stability as ceramic or metallic materials. The influence of different low-temperature sterilization techniques on the biocompatibility of a polymeric implant material was shown in own results [31].
A main scientific topic of our own group is the cell- and histocompatibility testing of an elastic degradable AB-copolymer network [32] in vitro and in vivo, which seems to be appropriate for the reconstruction of pharyngeal defects due to its physiochemical characteristics [33-35]. An adequate chemical, enzymatical, bacterial, and mechanical stability of the polymeric material is a requirement for the pharyngeal reconstruction by biomaterials.

Material and methods

Samples of an ethylene-oxide (EO) sterilized polymer (diameter 10 mm, 200 µm thick) were used for the reconstruction of a standardized complete defect of the gastric wall in male rats in a prospective study. Animal experiments have been approved by Regional Council Tübingen, Germany (Number 729) and were performed in accordance with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and to the regulation of the animal protection law in Germany. Guidelines for the “Principles of Laboratory Animal Care” have been observed.

The implantation material was a degradable polymer network based on oligo(epsilon-caprolactone) dimethacrylate as crosslinker and n-butyl acrylate as co-monomer was introduced. The anatomical localization of the stomach was used to test the stability of the implant material under extreme chemical, enzymatical, bacterial, and mechanical load. In the implantation group (n=42) the radical defect of the gastric wall was closed with the polymer network. 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 included 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. Essential parameters were the local
tight closure between the polymer and surrounding tissues. To test the impermeability between the implant material and adjacent gastric wall the intragastric pressure was measured after explantation of the stomach with maximal dilatation of the stomach by air insufflations. The measurement of the intragastric pressure was conducted in the same way in the control and the baseline group and statistical evaluation was performed.

Further decisive parameters were histological findings of tissue regeneration. Histological investigations were performed after 1 week, 4 weeks and 6 months in the implantation, control and baseline group. Removed tissue were fixed in 4% formalin with following paraffin embedding. 10 µm paraffin sections were performed at a microtome. Sections were stained according to the histological standard procedure with hematoxylin and eosin, H.E. staining [36]. Microscopic evaluation of the H.E. stained sections were done with special focus on the differentiation between tissue regeneration and scar formation.

To estimate systemic responses to inflammation the concentration of leucocyte in blood was measured by DxH 800 Hematology Analyzer, Beckmann Coulter, Germany.

Results
The surgical procedure and the postoperative course were without complications in all animals of the implantation and control group (n=63). Gastrointestinal complications like fistula, perforation or peritonitis did not occur in any animal of the implantation group (n=42) and the control group (n=21). A liquid- and gas-tight anastomosis between the polymer and the adjacent stomach wall existed in all animals of the implantation group. To test the impermeability between the implant material and adjacent gastric wall the intragastric pressure was measured after explantation of the stomach with maximal dilatation of the stomach by air insufflations (Figure 1). The
measurement of the intragastric pressure was conducted in the same way in the control and the baseline group and statistical evaluation was performed.

**Figure 1**: Stomach after 1 week of implantation time. The polymer implantation site is marked by arrows. A tube was inserted in the duodenum to insufflate air. The pressure was measured with a pressure probe in the esophagus.

The intragastric pressure by air insufflation was measured in the implantation, control and baseline group after 1 week, 4 weeks and 6 months. According the age of the animals and the increase of size of the internal organs the lowest values were found after 1 week and the highest values after 6 months (Figure 2). No statistical significant differences were found between the implantation, control and baseline group at the different time points (Figure 2).
Figure 2: Measurement of intragastric pressure (mm Hg) after maximum of air insufflation in the implantation, control and baseline group after 1 week, 4 weeks and 6 months. Reproduced with permission of Walter de Gruyter from [37]; permission conveyed through Copyright Clearance Center, Inc.

Histologically, a regeneration in the former defect zone with glandular epithelium was found after 4 weeks of implantation time (Figure 3). In the control group a scar formation was detectable 4 weeks after primary wound closure (Figure 4).
**Figure 3**: Histological findings (H.E. staining) after 4 weeks of polymer implantation. The former defect zone is marked by arrows. Regeneration with glandular epithelium was found in the defect zone.

**Figure 4**: H.E. staining in the control group 4 weeks after primary wound closure. In the area of primary wound closure scar formation was detectable in all animals. The scar formation is marked by arrows. The glandular tissue is adjacent to the scar formation and marked by stars.
In all three groups the highest concentration of leucocytes were found after 1 week of implantation time in the implantation and control group respectively time of observation in the baseline group. After 1 week the concentration of leucocytes showed the highest values in the control group and after 4 weeks in the implantation group. In all three groups a decrease of the concentration of leucocytes dependent on the duration was detectable (Figure 5).

Figure 5: Concentration of leucocytes (G/L) in the implantation, control and baseline group after 1 week, 4 weeks and 6 months. No statistically significant differences were found between the three different groups.

Discussion
Pharyngocutaneous fistulae are a serious complication after surgical oncological procedures in head and neck surgery. If patients need a surgical procedure after a
primary radiation therapy because of tumor recurrence in head and neck area there is a high risk for pharyngocutaneous fistulae [38]. Radiation therapy leads to reduced cellular oxygen concentration, increased apoptosis and reduction of capillaries in the irradiated tissue [39,40]. These mechanisms generate a disorder of the cellular metabolic balance and an impairment of cellular repair mechanisms. Clinical manifestations of these processes are chonic wounds and pharyngocutaneous fistulae in the head and neck area. Patients with pharyngocutaneous fistulae have a higher morbidity and mortality and their quality of life is distinctly reduced [41]. Oncological goals can not be reached when a radiation therapy can not be started because of pharyngocutaneous fistulae.

The biocompatibility and biofunctionality of implant materials are a basic requirement for their biomedical application. Novel biomaterials require a very complex testing of the materials in vitro and in vivo. Their degradation products have to be non-toxic and should not cause substantial changes in the pH value of the microenvironment of the implant.

The biocompatibility of the used polymeric material was studied in comprehensive in vitro investigations [42]. An excellent histocompatibility of the material was also shown after subcutaneous implantation in an animal model [43]. Furthermore, the material performed well in the chorionallantoismembrane assay. This assay is a very sensitive test to investigate the influence of a polymeric degradable material on the angiogenesis in vivo. The vascularisation of the chorionallantoismembrane was not influenced by the polymeric material. Based on the available results a suitable biocompatibility and biofunctionality of the novel polymeric material can be assumed.

Beyond biocompatibility, biomaterials in medical applications are closely in contact with biological systems, and their physical and chemical properties have been viewed as
important regulators for cell function and tissue regeneration [44,45]. The interaction between cells and biomaterials is complex and dynamic. A review is given in the literature [44]. Cells interacting with biomaterials are able to sense the materials’ inherent properties, such as substrate stiffness, surface topography, as well as functions e.g. chemical functionality, or biodegradability [44,46], and translate those cues into intracellular signaling activities that influences specific cell functions [47]. Cells are capable to secret extracellular matrix [48] with remodeling their own microenvironment. Or cells produce specific enzymes to degrade materials [49] releasing more degradation products. Thus, understanding of how inherent material properties regulate cell functions represents a tremendous opportunity to develop the next generation of dynamic biomaterials [47].

Biomaterials require characteristics that allow their integration into the surrounding tissue without eliciting an overshooting foreign body reaction (FBR). One of the major current goals of biomaterial research is therefore to understand, predict and intentionally influence the reaction to a biomaterial. A review about the pathology of the FBR against biomaterials is given by Klopfleisch et al. [33].

A lot of clinical research is going on in the use of synthetic meshes and biological scaffolds for abdominal wall repair [50-53]. Synthetic meshes provide adequate mechanical support but have significant drawbacks like seroma formation, adhesion to viscera, stiffness of abdominal wall and infection. Biologic scaffolds promote stem/progenitor cell recruitment and have enough mechanical strength for abdominal wall repair. But many concerns remain about the use of these scaffolds in the clinic due to the higher costs compared with synthetic meshes and having the same recurrence rate [53].
In our animal model the mortality of the gastric breakdown of the degradable polymer and/or sutures and fistula implying local or generalized peritonitis are comparable to the mortality of insufficiencies and salivary fistula of the pharynx. The adequate chemical, enzymatical, bacterial and mechanical stability of the polymeric material was shown under the extreme conditions of the stomach. The impermeability between the implant material and adjacent gastric wall, the missing gastrointestinal complications and the concentration of the leucocytes suggest that the polymer network did not induce systemic inflammation. After 4 weeks and 6 months of implantation time the former defect zone was replaced by histological regular built glandular tissue. The polymeric material seems to be an alloplastic splint for the tissue remodeling. In further investigations the mechanisms of the integration of the biomaterial in surrounding tissue as well as the degradation of the polymer and the process of the tissue remodeling should be analyzed with special focus on the interaction between cells and biomaterials with surface modifications. In a next step the question has to be answered if the polymeric material also induces tissue remodeling after reconstruction of the pharyngeal wall in the upper aerodigestive tract.

**Conclusion**

Mucosal reconstruction of the upper aerodigestive tract requires an adequate enzymatical, chemical, bacterial and mechanical stability of the implant material as well as a wound healing with fast integration of the material in surrounding tissue to avoid fistulae. While a lot of progress has been made in polymer chemistry in the last couple of years, substantial additional effort is required to move this technology to a clinical application.

The progress in several 3D printed technologies has transformed craniofacial reconstruction over the last 2 decades among other medical fields. These techniques
facilitate craniofacial bone reconstruction and preliminary soft tissue reconstructive efforts [54]. The progress in stem cell technology expect further development of novel therapeutic options with reconstruction of different tissues based on the principles of tissue engineering [55,56]. Over the past 10 years, robotic technology has had a significant impact on minimally invasive surgery. Transoral robotic surgery utilizing the da Vinci robotic system has opened a new era for minimally-invasive surgery in otolaryngology, head and neck surgery [57]. Robotic surgery is just beginning to have an impact on the field of plastic surgery. As this technology continues to advance in and out of the operating room, it will play an increasingly prominent role in our specialty and in our lives [58,59].

The reconstruction of the upper aerodigestive tract after tumor resection by polymeric materials instead of myocutaneous flaps would be a paradigm shift in oncological head and neck surgery. A major aim would be a better functional outcome concerning verbal communication and deglutition, reduced time of surgery and anesthesia because of missing need of preparation of myocutaneous flaps and reduced stay in hospital especially if the risk of pharyngocutaneous fistula can be reduced.

In a next step the copolymer network should be used for the pharyngeal reconstruction in a large animal study to test the material under pharyngeal conditions in vivo.
Acknowledgements

This work was financially supported by the Helmholtz-Association through program-oriented funding and by the German Federal Ministry of Education and Research (BMBF) through BioFuture grant no. 0311867.

References:


