Thieme

Regeneration – A New Therapeutic Dimension in Otorhinolaryngology

Authors
Nicole Rotter[1], Marcy Zenobi-Wong[2]

Affiliations
1 Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital of Mannheim, Germany
2 ETH Zurich, Tissue Engineering + Biofabrication, Zurich, Switzerland

Key words
Regeneration, regenerative medicine, tissue engineering, otorhinolaryngology

Bibliography
DOI https://doi.org/10.1055/s-0043-122309
Laryngo-Rhino-Otol 2018; 97: S200–S213

ISSN 0935-8943

Correspondence
Prof. Dr. med. Nicole Rotter
Department of Otorhinolaryngology, Head and Neck Surgery
University Medicine of Mannheim
University Hospital of Mannheim
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Germany
nicole.rotter@umm.de

ABSTRACT

Regeneration as a therapeutic principle and regenerative medicine in general are promising new strategies to add new therapeutic dimensions to our current treatment options. Currently, reconstructive surgery, drugs, and implants, including the cochlear implant, can replace the functions of damaged tissue. By contrast, regenerative therapies aim at the replacement of damaged tissues themselves while at the same time replacing their lost tissue function. In this review article, new technologies, including three-dimensional bioprinting and the application of decellularized tissues as biomaterials, are introduced and explained. A summary of current preclinical and clinical regenerative studies in otorhinolaryngology complements these basic aspects.

Contents

ABBREVIATIONS

ADSC	Adipose-derived stem cells
b-FGF	Basic fibroblast growth factor
ECM	Extracellular matrix
NIH	National Institute of Health
PEG	Polyethylene glycol
PRP	Platelet-rich plasma
SVF	Stroma vascular fraction

1. Regenerative Medicine

1.1 Principles of regeneration

Regeneration is defined as the ability of an organism to replace lost tissue and organs. The term should be limited to mechanisms that recapitulate processes during embryogenesis and fetogenesis [1]. Whereas in humans and most mammals this ability is mostly lost and is restricted to certain tissues, including bone marrow, gastrointestinal mucosa, liver, and skin [1], axolotls, Mexican salamanders, for example, are able to regenerate entire extremities. Recently, relevant mechanisms of this regeneration were determined [2]. It is currently assumed that various progenitor cells with defined regenerative potential are responsible for this form of regeneration [3]. However, macrophages also appear to play a key role in this process [4].

Following an injury in humans, inflammatory processes occur and scars develop. In general, the original tissue function is at least partly lost because the scar tissue is not identical to the original tissue. Additionally, the immune system and remodeling of the extracellular matrix (ECM) play a crucial role in regeneration in other organisms, including, for example, the axolotl. An increasing and better knowledge of these processes will most probably influence the development of regenerative strategies [5].

1.2 Introduction to regenerative medicine

The term of regenerative medicine and thus the use of the term regeneration as a therapeutic principle has in the meantime become accepted and is considered as one of the most promising fields of modern biomedicine. However, to date, no standardized definition exists. The NIH defines regenerative medicine as the "process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects" (https://report.nih.gov/NIHfactsheets/ViewFactSheet.aspx?csid=62). Regenerative medicine has the potential to solve the problem of the shortage of organs needed for organ transplantation [6].

Occasionally, this definition is even extended so that single technologies, including tissue engineering and the therapeutic application of stem cells, represent only some aspects of regenerative medicine. In addition, medical devices may be able to induce regenerative mechanisms and in specific cases even be a regenerative therapy. Furthermore, gene therapy is included in the notion of regenerative medicine when it enables the activation of regenerative mechanisms.

Therefore, regenerative medicine is entirely different from common therapeutic procedures that simply replace the tissue function but not the tissue itself. One obvious example is the cochlear implant. The cochlear implant is an extremely successful prosthesis that is able to replace the function of deficient hair cells by directly stimulating the auditory nerve. The cochlear implant replaces the function of the inner ear but not the inner ear itself. By contrast, regenerative therapy for inner-ear hearing loss and deafness would replace deficient or lost hair cells either by applying gene therapeutic approaches, by inducing regenerative mechanisms, or by differentiating still-existing or externally-applied cells into hair cells.

In otorhinolaryngology, tissue defects after trauma, tumor resection, or in the context of congenital defects also need to be restored. Furthermore, the natural ageing process leads to changes, including the decrease of the hearing threshold, that require treatment. To date, in addition to surgical and pharmaceutical therapies, particularly with regard to otology, hearing aids and prostheses are available that may treat such alterations. However, nearly all fields of otorhinolaryngology might also be the objective of regenerative, therapeutic approaches. They range from the area of otology, where currently prostheses are used very successfully to restore hearing, to reconstructive facial surgery, where currently highly complex surgical procedures are applied for reconstruction. These approaches include the application of local flaps and grafts, the application of pedicled flaps, and microvascular transplants as well as facial transplants [7], which was first performed successfully in 2005. Since the first operation, only 35 further cases of facial transplants have been described in the literature [8]. Innovative methods based on decellularized tissue and regenerative therapy strategies might provide an alternative in this field [9].

In the following sections, 2 important technologies from the field of regenerative medicine, namely three-dimensional (3D) bioprinting and decellularized natural materials, and their applications, will be presented.

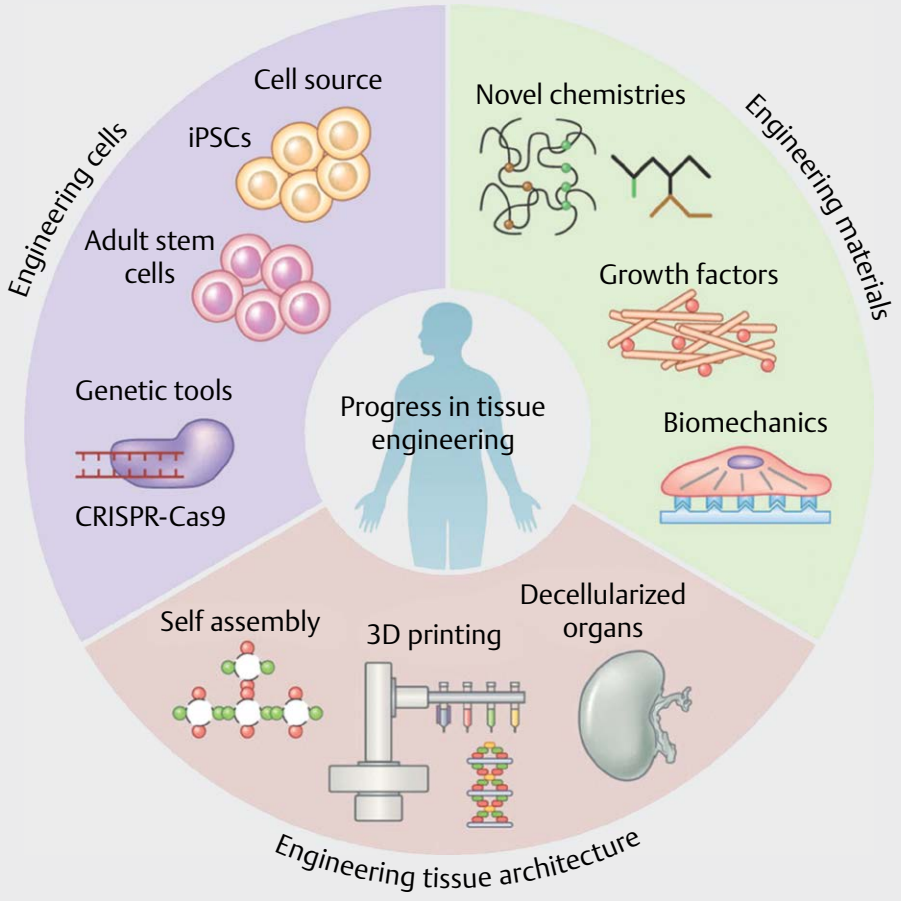
2. 3D Bioprinting

Tissue engineering has undergone tremendous advances due to the identification of novel stem cell sources and gene editing technologies as well as through the development of smart, responsive and cell-instructive materials. Perhaps the biggest leaps made in the field are being achieved through the combinations of these tools using advanced fabrication techniques such as bioprinting (▶ **Fig. 1**) [10]. By means of 3D bioprinting, cell suspensions may be printed layer-by-layer with biomaterials and thus highly complex three-dimensional structures can be created [11]. The potential advantages of bioprinting for reconstructive surgery include reduced donor site morbidity, reduced surgery time and improved aesthetic outcome.

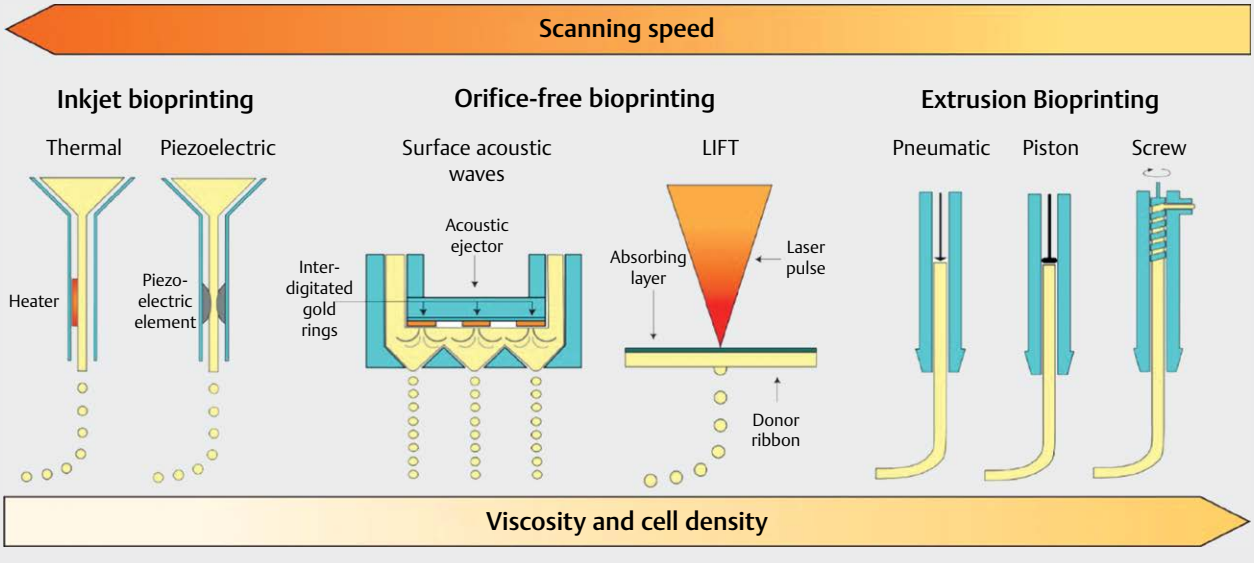
2.1 Bioprinting techniques and bio-ink

Bioprinting differentiates itself from conventional 3D printing in that the bioinks are solutions of hydrated polymers which can undergo crosslinking at physiologic conditions in the presence of cells. The 3D model which is printed can be designed from a photogrammetric scan of the patient or through reconstruction of MRI or CT data. Companies like Materialise (http://www.materialise.com) have specialized in producing accurate 3D models for surgical planning and clinical implants and prostheses. 3D models fabricated with bioprinting use one of 3 processing methods (▶ **Fig. 2**). In laser-assisted bioprinting (▶ **Fig. 2**, middle), a pulsed laser is positioned over an energy-absorbing layer, causing drops of cell-containing bioink to be deposited onto a substrate. Likewise in inkjet printing (▶ **Fig. 2**, on the left), minute droplets of hydrogels and cells are pulsed onto a substrate via the thermal or acoustic vibrations. In the most common method for printing larger, clinically-relevant structures (▶ **Fig. 2**, on the right), microextrusion is used to deposit strands of bioink onto the substrate, the flow of which is controlled by pressure or the movement of a mechanical screw (▶ **Fig. 2**). The properties of the bioinks for these 3 methods vary considerably. Materials for inkjet printing and laser-induced printing have generally lower viscosity and cell content, whereas bioinks for microextrusion require viscous solutions and can contain high densities of cells [12].

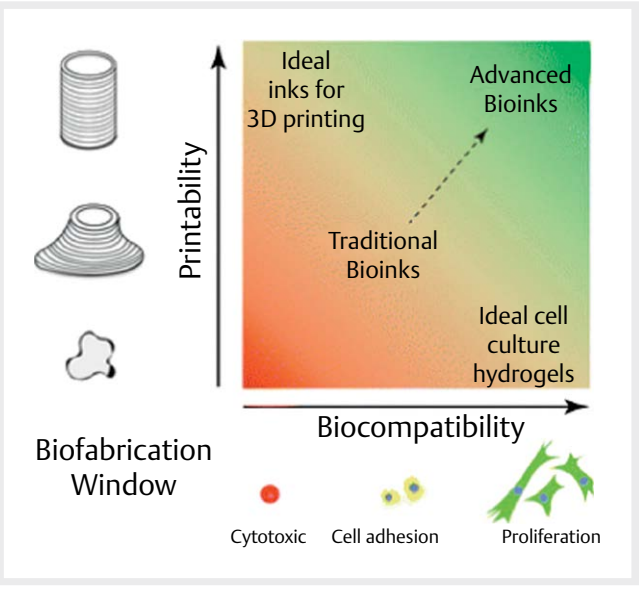
The success of bioprinted organs is highly dependent on the biological and rheological properties of the bioink. At the very least, an



▶ **Fig. 1** Important developments that promote the progress of regenerative medicine. Courtesy of [10].



▶ **Fig. 2** Overview of different 3D-bioprinting procedures. Courtesy of [12].



▶ **Fig. 3** Description of the so-called biofabrication window. Courtesy of [13].

ink must simultaneously provide excellent cytocompatibility and good printing resolution. This so-called bioprinting window has been non-trivial to achieve [13] (▶ **Fig. 3**). High water content hydrogels are an excellent mimic of the native cartilage matrix and residing cells can produce large amounts of extracellular matrix proteins. Hydrogels however do not print with good shape fidelity and are very weak and brittle. Alternatively, many materials with excellent printability derive these properties from high polymer content and/or high crosslink density which inhibits diffusion of nutrients and results in poor cell viability [13] (▶ **Fig. 3**).

To address this problem, a popular approach is to strengthen the properties of hydrogels through the coextrusion of a thermoplastic, stiffer material [14, 15]. In addition, there is a concentrated research effort to develop more advanced bioinks. The biological properties of bioinks can be augmented through the addition of decellularized matrix particles [16]. Likewise particles can enhance the mechanical properties of inks by serving as crosslinking nucleation sites [13].

2.2 Bioprinting techniques for the head and neck

Advanced manufacturing or 3D printing has already made inroads in maxillofacial reconstruction [17] but bioprinting approaches are still largely investigated at the research level [18]. Due to its unique and complex contours which are critical for the aesthetic appearance, the human auricle has been a favorite target for 3D printing [141]. Already surgical planning tools for auricular reconstruction have been utilized [14]. Many bioprinting approaches using a number of cell types (auricular chondrocytes [19], mesenchymal stem cells [20], induced pluripotent stem cells [21], and materials (nanocellulose and alginate)), (▶ **Fig. 4**, bottom [22, 23]) have been reported. Pure hydrogel ear constructs are soft post-printing and required in vitro maturation to increase tissue properties. Thermoplastic reinforced materials allow sufficient strength to resist skin contraction during implantation and the reinforcement can fill the entire auricular shape, or be used to strengthen individual deconstructed modules corresponding to for example the back plate, helix/tragus and crux/antitragal regions (▶ **Fig. 4** – top [142]).

In summary, bioprinting for craniofacial reconstruction has great promise to make more functional, living, patient-specific grafts with improve clinical outcome. However there are currently no bioprinted products on the market and few tissue engineered products which are commercially successful. The regulatory and financial challenges surrounding these complex combination products are considerable [24].

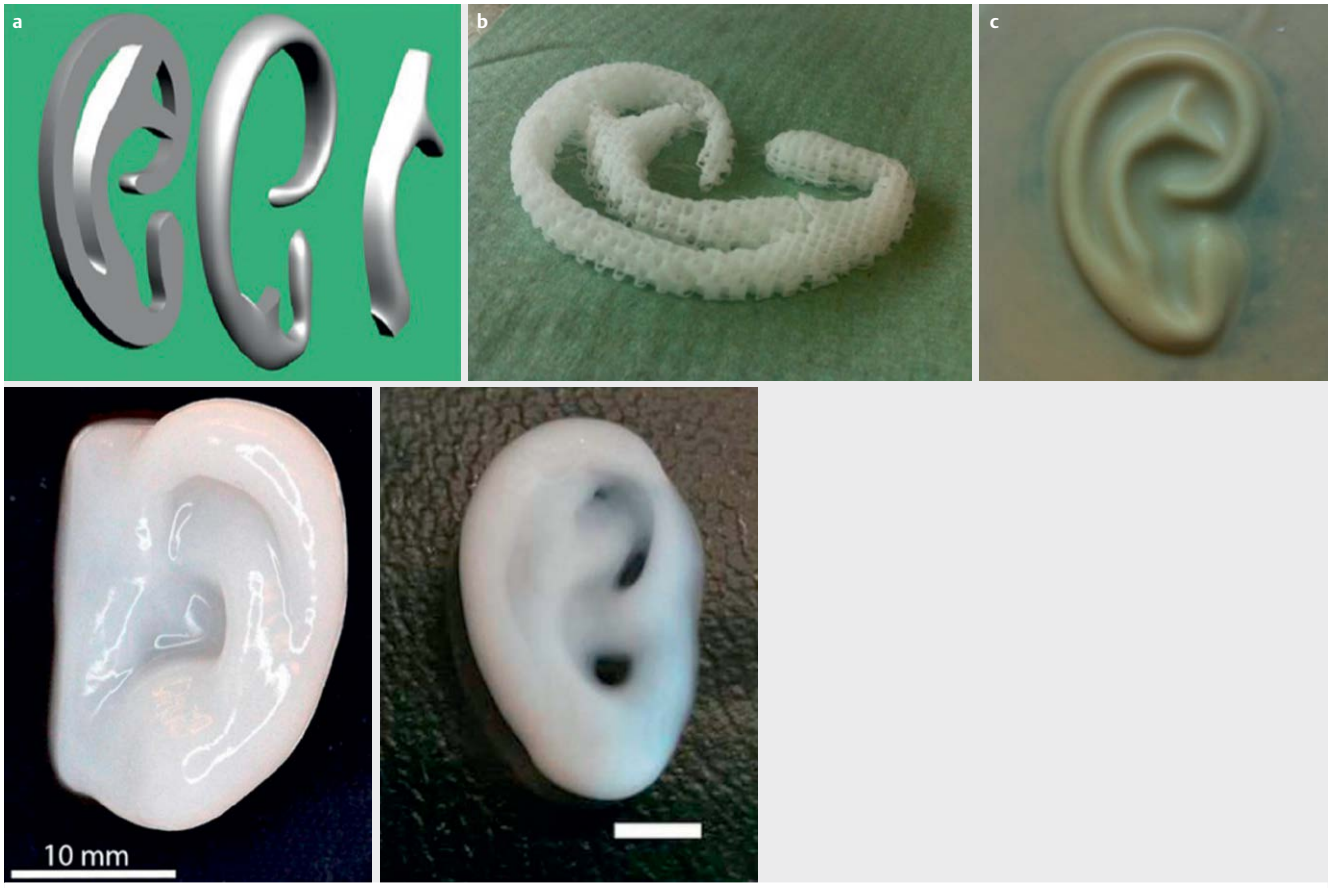
3. Decellularized Scaffold Materials

Scaffold materials are important components of in vitro and in-situ tissue-engineering techniques and of regenerative medicine in general. They provide mechanical stability and the specific shape for the tissue or organ that requires replacement. At the same time, they are expected to allow differentiation of the cells and nutrient transportation. The requirements of scaffolds are manifold and depend on the specific application [25, 26]. Generally, a distinction is made between artificial and natural biomaterials [27–29]. In recent years, biological scaffolds have been successfully developed based on decellularized tissue, also termed bioscaffolds, and applied both preclinically and clinically [30]. The main advantage of decellularized tissues is that they preserve the natural complex structure of the ECM of the original tissue and thus represent an excellent basis for in vivo colonization with differentiated local and progenitor cells; furthermore, they contain numerous signal molecules that may induce functional tissue remodeling [30]. These materials and their modifications have the potential to completely change current strategies of tissue regeneration, also because of their specific interaction with the immune system [31, 32] (see also chapter 3.2).

3.1 Basics and decellularization

The ECM consists of structural and functional molecules that are produced and secreted by local cells [1]. It is now well known that the ECM does not only create the structural preconditions but also contains extensive biological information [33]; and is itself actively responsible for the structural and functional alterations of the cells within the ECM. During development and growth, but also as a response to tissue damage, these processes are activated [30]. The ECM contains among others collagens, glycoproteins, glycosaminoglycans, proteoglycans, adhesion molecules, growth factors, chemokines, and cytokines [30]. The essential role of those proteins becomes clear from the fact that mutations that inactivate the function of single proteins, for example, laminin and collagen, are frequently lethal [34]. The ECM proteins as an important part of the so-called micro-environment are able to influence the differentiation of cells, including, in particular, stem cells [35]. In this context, the term stem-cell niche is also used [35]. Furthermore, this micro-environment influences the immune system and thereby the activity and function of macrophages, which was recently confirmed [31]. Based on this knowledge, new biomaterial-based therapies can be developed that may induce pro-regenerative immune responses and thus the desired tissue regeneration [31].

By means of various chemical, physical, and enzymatic methods, the local cells can be removed from tissue and organs, which is ter-



▶ **Fig. 4** Auricle and parts of auricles produced by means of bioprinting procedures using different materials. Top: courtesy of [142]; bottom right: courtesy of [22]. Copyright 2015 American Chemical Society, bottom left: courtesy of [23].

med decellularization [30]. Currently, it is possible to decellularize nearly all tissues and organs and thus to obtain tissue-specific scaffolds [36]. In 2011, Badylak used the term of (re)constructive tissue modeling as defining the creation of functional location-specific tissue by means of decellularized materials [37].

3.2 Role of macrophages

The role of macrophages as a relevant cellular component of regenerative mechanisms was discovered in recent years among others in the context of the regeneration of the extremities of the axolotl [4]. Additionally, macrophages play a key role in the regeneration of the zebrafish tail [38]. The role of macrophages in human wound healing is well known. Macrophages migrate to the site of the damage, clean the wound by phagocytosis, and initiate scarring.

Nonetheless, the roles of macrophages are increasingly analyzed in the context of the integration of biomaterials from decellularized tissue and they are considered to be relevant for regenerative medicine [39]. The positive aspects of macrophage activation, in particular, have been known for some time, whereby the shift of the pro-inflammatory M1 phenotype to the anti-inflammatory or remodeling M2 phenotype is a major aspect for functional tissue regeneration in contrast to scarring [39]. These findings serve for the production of biomaterials that may induce a regenerating phenotype instead of long-lasting inflammation. In this sense, they are relevant for the further development and modification of biomaterials that are essential, in particular, for the regeneration of supporting tissue, including tendons, bones, and cartilage.

4. Regenerative Medicine in Clinical Routine

4.1 Overview

Although regenerative procedures are increasingly applied in clinical trials, they are only rarely found in clinical routine [40].

The manufacturing of cartilage tissue by means of tissue-engineering procedures is one of the most developed fields of regenerative medicine. In orthopedics, autologous chondrocyte implantation (ACI) and matrix-based autologous chondrocyte implantation (MACI) are already established in clinical routine. In 1994, Brittberg et al. were the first to publish this procedure, in the New England Journal of Medicine. During the last 20 years, it has proven to be a significant clinical option [41–44]. In the meantime, it has become an alternative for traumatic defects, particularly in younger patients. Because the application of chondrocytes from the joints displays the relevant disadvantage of causing secondary problems in the area of the donor site, currently, nasal chondrocytes have become the focus of interest [45]. Nasal chondrocytes derive from the neural crest [46]. Different investigations demonstrated that nasal chondrocytes are also able to display their effect, in particular, the synthesis of extracellular cartilage matrix, in other locations and are thus suitable as a possible cell sour-

ce for transplantation [46, 47]. A clinical phase I study [45] has already been conducted that confirmed these findings also in the clinical practice. Currently, a larger phase I/II trial has started in Basel, Switzerland, which is expected to confirm these results and the effectiveness of therapy in a larger patient cohort. In a clinical phase I trial, nasal chondrocytes have also been applied for the reconstruction of the alar lobule [48]. In this study, nasal septum chondrocytes were pre-cultured on a collagen fleece made of type I collagen and then inserted as an alar lobule transplant in combination with a forehead flap for reconstruction of the nostril. Because for transection of the forehead flap and refinement and optimization of the appearance a second and mostly even a third intervention was always required, tissue could be collected from the reconstructed area for analysis. Hereby, tissue regeneration could be proven histologically.

4.2 Lacking availability of regenerative therapies in clinical routine

A comparison of scientific publications with our clinical practice clearly shows that numerous experimental and preclinical studies on a wide range of topics are published without the possibility of applying them in clinical practice. A significant number of review articles deal with the question of why commercialization of such therapies is so difficult [40, 49, 50]. In general, the obstacles are found in the clinical, commercial, and regulatory sectors [50]. Frequently, preclinical data allow only insufficient transferability to humans [50]. Study design and ethical and safety concerns are the issues focused on in the clinic [51, 52], whereas commercialization is impeded by increasing costs and a high product-development risk [53]. Continuously higher safety level requirements and efficiency standards of a therapy as well as different regulations in different countries are relevant problems in the legal context [54]. Further important specific factors that have been identified, including the insufficient support of preclinical and clinical trials, a lack of knowledge by basic and clinical scientists about regulatory aspects that have to be observed when conducting studies from a commercialization aspect, the uncertain financial reimbursement of innovative therapies, and the production and upscaling aspects that are essential for commercialization [40, 49]. One crucial factor for success for all actors in this field is to be aware of all these obstacles and to address them specifically already in the very early stages of research and development. This is only possible based on close interdisciplinary cooperation between industry and the regulatory institutions.

In addition to the above-mentioned factors, a fundamental rethink for the whole field of regenerative medicine is currently required [55]. Many experimental investigations do not or only partly include the basic vascular, neural, and lymphatic provision; frequently even the local microenvironment is not sufficiently considered [55]. Furthermore, immunological factors are frequently bypassed by using immune-incompetent animals. However, these factors are essential and of crucial relevance for clinical application. In future, it will be important to perform regenerative medicine even more within an interdisciplinary framework than is currently the case. Knowledge in the fields of developmental biology and immunology, as for example the role of macrophages in the limb regeneration of salamanders [2, 4], is only one such example. A close cooperation with developmental biology and immunology will be essential for regenerative medicine and is relevant for its viability.

5. Regenerative Procedures in Otorhinolaryngology – State-Of-The-Art

In the following, the focus will be placed on areas where clinical applications of regenerative therapies have already been published or preclinical trials approach clinical application. In addition, the above-mentioned fields of 3D bioprinting and decellularized scaffolds will be described in more detail, provided that they are relevant for the respective area. An overview of clinical studies of the indicated clinical applications is summarized in ▶ **Table 1**.

5.1 Rhinology and plastic-reconstructive surgery

5.1.1 Nose

Defects in the region of the nose may be congenital, traumatic, or iatrogenic. In rhinology and plastic-reconstructive surgery of the head and neck, numerous clinical studies applying regenerative procedures for the reconstruction of cartilaginous tissue of the nose have already been conducted and published. They start with the use of autologous chondrocytes for augmentation of the nasal dorsum, as first published by Yanaga et al. in 2004 [56]. In this publication, 8 patients were described from whom autologous chondrocytes were isolated from the cartilage of the cavum conchae and amplified. Subsequently, the generated gel-like suspension was injected into the nasal dorsum and in one case into the chin for augmentation. The assessment of the outcome was mainly performed macroscopically and in one case by means of magnetic resonance imaging. In another study from 2006 [57], further results achieved with this methods were published. In 32 patients, a suspension of amplified auricular chondrocytes was used for augmentation of the nose and other locations. The outcome here was also assessed mainly macroscopically. In 8 patients, a biopsy taken from the transplanted tissue suggested the presence of cartilaginous tissue. Significant limitations of these studies include insufficient study design without control groups or standardized evaluation and a lack of a description of the cell-culture technique, which makes repetition of the investigations impossible. Therefore, it is clearly not possible to draw further conclusions from these studies, even though Yanaga and his team applied this technique again in another study with 18 patients in 2013 [58]. This time, a slight modification of the cell-culture method was performed and the tissue was first transplanted into the abdominal wall. After approximately 6 months, the transplanted tissue, now surrounded by fatty tissue, was used for augmentation of the nasal dorsum and the chin in special cases with particularly thin skin. However, to date, no publications by other authors using this technique are known. Yanaga and co-workers described this technique in further publications, including for the creation of auricular cartilage for the treatment of microtia (see chapter 5.1.2). In 2017, a case report was published by Ceccarelli et al. [59], who applied a micrografting technique patented for the treatment of chronic wounds ("Rigenera®") [60] in open septorhinoplasty that required insertion of spreader grafts. Unfortunately, this publication also failed to clearly describe the methods and rationale.

A relevant, and thus important, progress was achieved in a study by Fulco et al. in 2014 that was published in the Lancet [48]. The aim of this phase I study was to investigate the safety and feasibility of their method. The alar lobule of 5 patients was reconstructed after tumor resection using cartilage tissue produced in vitro. Additionally,

▶ **Table 1** Different stages of the development of regenerative medicine in the head and neck regions.

	Case reports and case series	Phase I	Phase II/III	Commercial product	Routine
Cartilage, nose	Augmentation of the nasal dorsum (n = 8; n = 32) Yanaga, Japan [57] Spreader graft (?) (n = 1) Ceccarelli, Italy [60]	Reconstruction of lateral alar cartilage (n = 5) Fulco, Switzerland [48]	–	–	–
Cartilage, auricle	Partial and total reconstruction of the auricle (n = 12) Yanaga, Japan [66]–[67]	–	–	–	–
Facial nerve	– Facial nerve; lesion of a length of up to 3 cm Navissano, Italy (n = 7); NeuroTube [75] – Facial nerve Gunn, USA (n = 1); Avance [79] – Facial nerve – frontal branch Inada, Japan (n = 2); PGA collagen tube, no commercial product [77] – Chorda tympani Yamanaka, Japan (n = 3); PGA collagen tube, no commercial product [78]	–	–	e. g. - PGA; NeuroTube® – Collagen I: NeuraGen®, NeuroMatrix®, NeuroFlex® – NeuraWrat®, NeuroMend® – decellularized human allograft Avance®	–
Vocal folds	–	–	–	–	–
Larynx	–	–	–	–	–
Trachea	12-year-old child, compassionate use, Hamilton, UK [101–102]	–	–	–	–
Eardrum	Gelatine + b-FGF (n = 53), Kanemaru, Japan [104]	Gelatine + b-FGF (n = 11), Kanemaru, Japan [106]	Gelatine + b-FGF; ongoing according to [106]	Alloderm® Tutopatch® Audiomesh® Surgisis®	–
Mastoid	Kanemaru, Japan (n = 10) [115] Kanemaru, Japan (n = 26) [117]	–	–	–	–
Salivary glands	PRP + ADSC + SVF, intraglandular, Cornella, Italy (n = 1) [138]	Phase I/II study protocol, mesenchymal stem cells (n = 30), Gronhoj, Denmark [139]	–	–	–

a forehead flap or a nasolabial flap was used for the reconstruction of the outer skin. The cartilage tissue was obtained from the cartilage of the nasal septum, and chondrocytes were isolated and amplified in vitro and then cultured on a collagen I fleece (Chondro-Gide, Geistlich Pharma, Wullhusen, Switzerland). This collagen I scaffold had already been tested and approved for use in joints. In parallel, 2 scaffolds were cultivated. One scaffold was used for transplantation, the second for in vitro analysis so that an assessment of the in vitro chondrogenesis could be performed. After 6 months, the reconstructions were refined and at the same time tissue for histologic examination was obtained from the transplanted area. The study confirmed the safety and feasibility of this method. Furthermore, it was observed for the first time that in vitro-produced cartilage tissue was still present on the site of transplantation, even if the amount was highly variable. The secondary outcome parameters of patient satisfaction and stability of the alar lobule, as assessed by means of nasal-flow measurement, also indicated that this technique was an alternative for classic transplantation of septum or ear cartilage for the reconstruction of lateral alar cartilages. A controlled study verifying and refining these results is currently unavailable.

5.1.2 Auricle

Defects of the auricle may be congenital or occur after trauma or tumor resection. Despite a multitude of in vitro and animal experimental studies confirming the possibility to produce cartilage in the shape of a human auricle [61–65], currently, there are no high-quality trials that have applied this technique in clinical practice. Only Yanaga et al. used the technique described above (see chapter 5.1.1), where it was applied for nasal augmentation, in a modified way for auricle reconstruction [66, 67]. The authors isolated chondrocytes from the microtic auricles of 4 patients and used these cells to produce a cartilage matrix that developed 6 months after subcutaneous injection of the cells in the abdominal region. Subsequently, without an exact description of the technique, an auricular scaffold [68, 69] was shaped from this cartilage matrix and transplanted into the auricular area. According to the authors, 12 patients have now been treated, and up to 6 years after surgery no relevant resorption of the auricular scaffold has been observed [67].

In particular, for complex 3D structures like the human auricle, 3D bioprinting appears to be optimal for restoration. In an initial publication, the principle could be clearly demonstrated [23]. However, in addition to the 3D shaping, the surrounding skin frequently

represents a significant problem in auricular reconstruction, because in most cases the available skin is much thicker than the auricular skin. Therefore, a major objective is the creation of a vascularized composite graft from cartilage and skin.

The decellularization of ear cartilage might also be a pioneering innovation in the field of auricular reconstruction. Utomo et al. have already characterized in detail the decellularized human auricle [70]. However, own results (unpublished) reveal an insufficient stability of decellularized auricles after implantation in rabbits.

5.1.3 Facial nerve

Neural damage in the head and neck regions may be traumatic, cancer-related, but also iatrogenic. Frequently, the facial nerve is involved. The treatment encompasses end-to-end anastomoses when the defect length is relatively short, whereas for longer gaps that cannot be adapted tension-free, the application of autologous nerve transplants is the current gold standard [71]. The use of autologous nerve transplants is associated with donor site morbidity, including sensitivity deficits; furthermore suitable transplants are not always available regarding length and diameter [72]. With this background, regenerative procedures are considered as being a promising alternative for nerve repair [73]. In recent years, a multitude of new techniques have been developed for reconstructing nerve defects (nerve tubes). Some of them have reached the clinic, but without being extended into the clinical routine. These procedures pursue among others the principle of finding suitable tubes for the growth of the axons while at the same time impeding the ingrowth of fibroblasts from the environment [74]. For example, autologous veins have been used successfully as neurotubes, but they are not always available. Therefore, synthetic tubes are now a focus of research. Absorbable materials are preferred, because secondary interventions may be avoided for the removal of the non-absorbable materials. Polyglycolic acid, which has been used as a component of surgical suture material for many years, was approved as the first absorbable nerve transplant (NeuroTube, Synovis, Birmingham). In addition to others, Navissano et al. [75] reported the successful clinical application of NeuroTube in lesions of the facial nerve with gaps of up to 3 cm. Negative aspects are the price, the possibly too rapid resorption rate, and the risk of toxic metabolites [73]. In addition, tubes made of collagen I were applied in many preclinical investigations and in clinical studies, and appear to be equivalent to an as autologous nerve transplant for gaps of approximately 1.5–2 cm [73]. Currently, 5 collagen neurotubes are available for clinical use (NeuraGen, NeuroMatrix, NeuroFlex, NeuraWrap, and NeuroMend). Nonetheless, their application is not firmly implemented in clinical routine. Because the published trials do not provide consistent results, it remains unclear whether neurotubes are suitable for longer defects (> 1.5 cm), even if they proved to be equivalent to a nerve transplant for short gaps [76]. In 2007, Inada et al. applied a neurotube made of polyglycolic acid (PGA) and collagen I to repair the frontal branch of the facial nerve in 2 patients [77]. Furthermore, a small case series (n = 3) was published by Yamanaka et al., who successfully reconstructed the chorda tympani using a similar tube of PGA and collagen I [78]. Both products are not commercially available or approved in Germany. In a case report, Gunn et al. described the repair of the tympanic and mastoidal segments of the facial nerve using a decellularized human implant ("Avance") [79].

Decellularized nerve transplants are currently being evaluated in preclinical trials. The initial results indicate comparable outcomes with respect to autologous nerve transplants [80, 81]. The use of 3D-bioprinting techniques has been suggested for nerve regeneration, because of the excellent possibility to produce clearly defined tubes [82].

5.2 Laryngology and tracheal surgery

5.2.1Vocal cords

The vocal folds as a vibratory and complex multilayer part of the larynx are responsible for respiration and phonation. Biomechanical stress, smoking, inflammation, irradiation, or intubation may significantly disturb the function of the vocal folds and lead to a significantly impaired quality of life [83]. Voice therapy of various disorders is not always sufficient, but surgical treatment is always associated with the risk of additional scarring and further deterioration of the voice [84]. Therefore, the treatment of functional disorders and defects of the vocal folds is also an important aim of regenerative strategies. Research currently focuses on the application of bioactive factors, biomaterials, and stem cells [85–87]. The requirements of suitable biomaterials are extremely complex, because on the one hand mechanical stability for insertion into the larynx is necessary and on the other hand the vibratory ability of the vocal folds requires enormous flexibility. Hydrogels were evaluated several times regarding injection into the vocal folds [88], with materials like collagen and elastin play a key role as well as the combination with stem cells or fibroblasts from the patients' own vocal folds [89]. Stem-cell application can be performed by injection or mobilization of endogenous stem cells [87]. This procedure has already been investigated in animal models, in particular in cases of acute damage of the vocal folds. Clinical trials have not yet been performed

Like the application of decellularized vocal folds, 3D bioprinting has only been described in 3D bioprinting and decellularization of the entire larynx [90, 91].

5.2.2 Larynx

Because of the diversity of tissues in the larynx and the complex function for voice formation and swallowing, the creation of an artificial larynx is a considerable challenge. Currently, the restoration of laryngeal function after partial or total laryngectomy is only partly possible and is associated with major impairment for the patients. Hamilton and Birchall state in a recent review article that the treatment of laryngeal cancer will be crucially influenced by the developments in the field of laryngeal regeneration over the next 10 years [92]. Larynx transplantation is currently mainly a theoretical option that can only be applied in exceptional cases and that is not suitable for reconstruction after tumor surgery. However, it has been described twice in the literature [93, 94]. To create an artificial larynx, the production of various tissues, including cartilage, laryngeal muscles, and laryngeal mucosa, must be coordinated. These tissues have to be connected with the vascular and neural systems of the receiver to restore laryngeal function. A potential alternative is the decellularization of an allogenic larynx as a scaffold that could be seeded with different cell types [91]. One major advantage of this strategy is that the complex laryngeal shape and the various ECMs of the different tissues are available as sources for seeding. However, to date, no preclinical or clinical applications of this strategy have been pub-

lished. Another option for laryngeal reconstruction is the application of bioprinting strategies, of which the general principles were described in detail in chapter 2. However, for the larynx, no references are available, but an individualized tracheal stent made of polycaprolactone by bioprinting has already been successfully implanted [95]. Further progress in this field may well develop as rapidly as assumed by Hamilton and Birchall [92].

5.2.3 Trachea

In general, tumors and trauma, but also congenital lesions may lead to a situation where important parts of the trachea require reconstruction. Because resection and end-to-end anastomoses are only possible up to a length of approximately 5 cm in adults, the trachea is also an important focus of regenerative procedures [96, 97].

Even if the trachea was considered as the first organ that could be produced in vitro by means of stem cells [98], its attempted regeneration was a disaster within the entire field of regenerative medicine because, patients underwent surgery without sufficient preclinical data or a solid scientific basis [97]. The published data have to be considered as scientific fraud [99, 100]. Only one case of the successful application of a decellularized trachea seeded in vivo with autologous cells can be cited [101]. In this case, a 12-year-old child suffering from congenital long-segment tracheal stenosis was treated with a decellularized trachea [102]. The child has to date survived 4 years since this treatment, although multiple revisions have been necessary [101].

Even if decellularization and 3D printing are important approaches for tracheal reconstruction, especially for tracheal surgery, extensive and thorough experimental and preclinical data are essential before further clinical application.

5.3 Otology

5.3.1 Tympanic membrane

Defects of the tympanic membrane may occur during acute and chronic otitis media, but also after trauma. While acute tympanic membrane perforations have a very good rate of spontaneous healing, chronic defects require surgical treatment. Although this treatment is frequently successful when cartilage-perichondrium, perichondrium, or muscle fascia transplants with a low donor site morbidity are applied, nonetheless a surgical intervention is required under local or general anesthesia and is not always successful. For this reason, the tympanic membrane is also an objective of research in regenerative medicine; and cost-effective non-surgical therapeutic options are being investigated [103].

Already in 2011, Kanemaru et al. reported the successful clinical closure of perforations of the tympanic membrane in chronic otitis in more than 98 % of the patients [104]. In this study, the tympanic membrane was surgically restored and then a small defect-adapted block of gelatin with or without basic fibroblast growth factor (b-FGF) was applied and fixed using fibrin glue. Only the addition of b-FGF led to the high closure rates while only 1 of 10 perforations could be closed in the control group. Jackler called this development possibly the greatest progress in otology since cochlear implantation [105]. The results of the first study published by Kanemaru et al. were confirmed in a subsequent study in 2017 [106]. However, in this later trial, only 11 patients were treated. In the sense of a phase I study, first the safety of this therapy was analyzed without identifying therapy-induced adverse events. Long-term results have not yet been published. Furthermore, the study design, in particular of the 2011 trial, does not correspond to current standards of a clinical phase I study. Nonetheless, both clinical studies provided the first evidence that regenerative therapy might be suitable for the closure of tympanic membrane perforations. Another larger prospective randomized clinical trial was initiated that according to the authors is currently recruiting patients [106]. The technique to apply gelatin with b-FGF was furthermore employed by the authors to treat auditory meatus defects in 54 patients [107]. Unfortunately, the preclinical rationale of this study and its design is imprecisely described. In preclinical research, 3D bioprinting is also used to regenerate the tympanic membrane [108], which could be shown in the closure of chinchilla tympanic membrane defects. Even decellularized tissue has already been analyzed for tympanoplasty in preclinical studies and in some clinical trials [109, 110]. In particular, AlloDerm (LifeCell Corp., USA), which is a product of decellularized human skin, was demonstrated to be equivalent to temporalis fascia with regard to closure rates [109, 110] while at the same time requiring a shorter duration of surgery [111]. However, in Germany, AlloDerm is currently not available for tympanoplasty. An overview published by Kaftan presents further decellularized materials in detail [112]. Currently, these materials are not applied for tympanoplasty in Germany on a larger scale. Our own investigations revealed that the acoustic properties of decellularized cartilage tissue are comparable with human tympanic membranes and thin cartilage transplants [113]. However, this material is not currently available for clinical trials.

5.3.2 Mastoid

The mastoid is also a focus of regenerative medicine in otolaryngology [114, 115]. In addition to the Eustachian tube, pneumatized mastoid cells play a key role in pressure balance in the middle ear [116]. Their presence and function can impede the development of cholesteatomas and other chronic middle-ear diseases [115]. In a clinical study, 3D hydroxyapatite (3D-HA) was applied in 10 patients for reconstruction of mastoid air cells. After 12 months, in up to 60 % of cases, re-epithelized mastoid cells were found during second-look surgeries [115]. The authors postulate that in applying this method, cases of chronic otitis might be treated that otherwise could not be optimally treated. In another trial from 2013, Kanemaru et al. published a positive effect of this therapy on the function of the Eustachian tube [117]. In 26 patients, again 3D-HA was applied for regeneration of mastoid air cells in addition to conventional cholesteatoma treatment and tympanoplasty. In approximately 70 % of cases, an improved tube function could be confirmed intraoperatively compared to the pre-surgery situation, while this was only observed in approximately 13 % of the conventionally-treated patients. Furthermore, there are other preclinical studies in which other materials, including poly-D,L-lactide-poly-glycolic acid/polyethylene glycol (PLGA/PEG) [118] and polycaprolactone/β-tricalcium phosphate (PCL/β-TCP) [119], are used for reconstruction of mastoid air cells. Neither 3D bioprinting nor decellularized tissues have to date been used for mastoid reconstruction.

With regard to regeneration and preservation of hair cells, numerous experimental investigations have been conducted. Recently, pioneering publications on inner-ear regeneration have appeared [120,121]. They demonstrate that the therapeutic regeneration of

human hair cells might be a potential new way of treatment. Because another review article of this issue deals with treatment of the inner ear, this topic will not be considered here, rather we refer to the contribution of T. Moser and the references [120, 121].

5.4 Salivary glands

Xerostomia after radiation or radioiodine therapy is a serious adverse effect of these therapies and significantly impairs the quality of life of head- and neck-cancer patients. Currently, there is no causal therapy for xerostomia. Even highly improved irradiation procedures, including intensity modulated radiotherapy (IMRT) [122, 123], and the preventive application of amifostine [124] are unable to completely prevent xerostomia. Innovative radiation procedures that attempt to spare stem cell-containing glands [125] are not yet available in the clinical practice. Therefore, salivary gland tissue is also an important focus of regenerative therapy procedures. In addition to classic tissue-engineering approaches to produce glandular tissue in vitro, today stem cell-based procedures have come to the fore [126]. A relevant aspect of radiation- and radioiodine therapy-induced damage is the loss of acinar cells in addition to fibrosis, such that saliva secetion after these therapies is severely reduced [127–129]. Therefore, it may be beneficial that the original glandular structure is still present, and by means of stem cells the function of the salivary glands may be restored.

A large number of preclinical studies have been conducted in various animal models with different cell types that all revealed that stem cells after tissue damage (i. e. surgical trauma or radiation) migrate to the site of damage [130–133] and positively influence the tissue there. Additionally, a direct positive effect of stem cells could be confirmed in several animal models. The research team of Coppes from Groningen, The Netherlands, has provided basic explanations for the effective mechanism of stem cells originating from salivary glands. They convincingly showed that these cells are able to significantly increase saliva production [134–137]. In 2017, 2 studies were published that applied stem cells for the regeneration of radiation damage in patients for the first time [138, 139]. One of these studies is only a case report. The authors applied a mixture of platelet-rich plasma (PRP), adipose-derived stem cells (ADSC), and stromal vascular fraction (SVF) from autologous lipoaspirate to the parotid glands and the submandibular glands of both sides. After 31 months, no severe adverse events were reported and according to the authors, the patient wanted to further participate in the treatment. Important information with regard to the safety and effect of such therapy cannot be retrieved from this report about the application of the cell mixture in one patient [138]. Detailed planning of a phase I study is required, as described by authors from Denmark: They published the study protocol of a placebo-controlled, double-blind randomized phase I/II study that applies adult mesenchymal stem cells for the regeneration of radiation damage in 30 patients after radiation exposure (EudraCT, Identifier: 2014-004349-29; clinicaltrials.gov, Identifier: NCT02513238) [139]. The clinical results of the study are not yet available. It may be expected that also glandular stem cells will be applied in the near future in first phase I or phase I/II studies, so that salivary gland regeneration might be one of a few areas where the findings of preclinical investigations really do lead to clinical studies, even if it is not yet part of the clinical routine.

Based on the current development in the field of 3D bioprinting, cell- and biomaterial-based tissue-engineering strategies for salivary gland regeneration may also receive a new boost, because they may enable the production of complex 3D structures, including the salivary glands [140].

Conclusion

Whereas regenerative therapies for example in orthopedics are applied not only in clinical studies but also in clinical routine, only a few approaches have reached the level of clinical phase I studies in otorhinolaryngology, despite a variety of potential applications and a similar variety of preclinical studies. These include the application of tissue-engineered cartilage tissue for the reconstruction of the nose and of stem cells for salivary gland regeneration after radiation.

Significant obstacles to the clinical translation and subsequent extension into the clinical routine are the high costs that are associated with such individualized regenerative therapies. Even regulatory preconditions for clinical application frequently cannot be sufficiently fulfilled. Despite all these obstacles, regenerative medicine as innovative technology will fundamentally influence all areas of medicine, including the discipline of otorhinolaryngology, in the following years and decades. It is essential to discontinue unsuccessful strategies and to combine new findings from cell and developmental biology with the progress of immunology and new technologies, including bioprinting [55].

Acknowledgements

NR and MZW acknowledge funding from the Swiss National Science Foundation, grant no: CRSII5_173868.

Conflict of Interest

Prof. Dr. Marcy Zenobi-Wong is a founder and consultant for Auregen Biotherapeutics SA.

References

[1] Londono R, Badylak SF. Biologic scaffolds for regenerative medicine: mechanisms of in vivo remodeling. Ann Biomed Eng 2015; 43: 577–592

[2] Kragl M, Knapp D, Nacu E et al. Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 2009; 460: 60–65

[3] Voss SR, Epperlein HH, Tanaka EM. Ambystoma mexicanum, the axolotl: a versatile amphibian model for regeneration, development, and evolution studies. Cold Spring Harb Protoc 2009; 2009: pdbemo128

[4] Godwin JW, Pinto AR, Rosenthal NA. Macrophages are required for adult salamander limb regeneration. Proc Natl Acad Sci U S A 2013; 110: 9415–9420

[5] Atala A, Irvine DJ, Moses M et al. Wound Healing Versus Regeneration: Role of the Tissue Environment in Regenerative Medicine. MRS Bull 2010; 35: 8

[6] Orlando G, Soker S, Stratta RJ et al. Will regenerative medicine replace transplantation? Cold Spring Harb Perspect Med 2013; 3:

[7] Devauchelle B, Badet L, Lengele B et al. First human face allograft: early report. Lancet 2006; 368: 203–209

[8] Devauchelle BL, Testelin SR, Davrou J et al. Face graft? Extrapolation of facial allotransplantation to children. J Craniomaxillofac Surg 2016; 44: 925–933

[9] Duisit J, Maistriaux L, Taddeo A et al. Bioengineering a human face graft: the matrix of identity. Ann Surg 2017; 266: 754–764

[10] Khademhosseini A, Langer R. A decade of progress in tissue engineering. Nat Protocols 2016; 11: 1775–1781

[11] Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014; 32: 773–785

[12] Hölzl K, Lin S, Tytgat L et al. Bioink properties before, during and after 3D bioprinting. Biofabrication 2016; 8: 032002

[13] Chimene D, Lennox KK, Kaunas RR et al. Advanced bioinks for 3D printing: A materials science perspective. Annals of Biomedical Engineering 2016; 44: 2090–2102

[14] Melchels FPW, Blokzijl MM, Levato R et al. Hydrogel-based reinforcement of 3D bioprinted constructs. Biofabrication 2016; 8: 035004

[15] Visser J, Melchels FP, Jeon JE et al. Reinforcement of hydrogels using three-dimensionally printed microfibres. Nat Commun 2015; 6: 6933

[16] Pati F, Jang J, Ha DH et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 2014; 5: 3935

[17] Louvrier A, Marty P, Barrabe A et al. How useful is 3D printing in maxillofacial surgery? J Stomatol Oral Maxillofac Surg 2017; 118: 206–212

[18] Visscher DO, Farre-Guasch E, Helder MN et al. Advances in bioprinting technologies for craniofacial reconstruction. Trends Biotechnol 2016; 34: 700–710

[19] Kesti M, Eberhardt C, Pagliccia G et al. Bioprinting complex cartilaginous structures with clinically compliant biomaterials. Advanced Functional Materials 2015; 25: 7406–7417

[20] Daly A, Critchley S, Rencsok E et al. A comparison of different bioinks for 3D bioprinting of fibrocartilage and hyaline cartilage. Biofabrication 2016; 8: 045002

[21] Nguyen D, Hägg DA, Forsman A et al. Cartilage tissue engineering by the 3D Bioprinting of iPS cells in a nanocellulose/alginate bioink. Scientific Reports 2017; 7: 658

[22] Müller M, Öztürk E, Arlov Ø et al. Alginate sulfate–nanocellulose bioinks for cartilage bioprinting applications. Annals of Biomedical Engineering 2016, doi:10.1007/s10439-016-1704-5 1–14

[23] Markstedt K, Mantas A, Tournier I et al. 3D bioprinting human chondrocytes with Nanocellulose-Alginate bioink for cartilage tissue engineering applications. Biomacromolecules 2015; 16: 1489–1496

[24] Hourd P, Medcalf N, Segal J et al. A 3D bioprinting exemplar of the consequences of the regulatory requirements on customized processes. Regenerative Medicine 2015; 10: 863–883

[25] Kiyotake EA, Beck EC, Detamore MS. Cartilage extracellular matrix as a biomaterial for cartilage regeneration. Ann N Y Acad Sci 2016; 1383: 139–159

[26] Smith BD, Grande DA. The current state of scaffolds for musculoskeletal regenerative applications. Nat Rev Rheumatol 2015; 11: 213–222

[27] Hiew VV, Simat SFB, Teoh PL. The Advancement of Biomaterials in Regulating Stem Cell Fate. Stem Cell Rev 2017, doi:10.1007/s12015-017-9764-y

[28] Sampath U, Ching YC, Chuah CH et al. Fabrication of porous materials from natural/synthetic Biopolymers and Their Composites. Materials (Basel) 2016; 9: 12

[29] Edgar L, McNamara K, Wong T et al. Heterogeneity of scaffold biomaterials in tissue engineering. Materials (Basel) 2016; 9: 5

[30] Swinehart IT, Badylak SF. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. Dev Dyn 2016; 245: 351–360

[31] Sadtler K, Estrellas K, Allen BW et al. Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science 2016; 352: 366–370

[32] Badylak SF. Tissue Regeneration. A scaffold immune microenvironment. Science 2016; 352: 298

[33] Bissell MJ, Aggeler J. Dynamic reciprocity: How do extracellular matrix and hormones direct gene expression? Prog Clin Biol Res 1987; 249: 251–262

[34] Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol 2010; 341: 126–140

[35] Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. Biochim Biophys Acta 2014; 1840: 2506–2519

[36] Cravedi P, Farouk S, Angeletti A et al. Regenerative immunology: the immunological reaction to biomaterials. Transpl Int 2017, doi:doi:10.1111/tri.13068

[37] Badylak SF, Brown BN, Gilbert TW et al. Biologic scaffolds for constructive tissue remodeling. Biomaterials 2011; 32: 316–319

[38] Petrie TA, Strand NS, Yang CT et al. Macrophages modulate adult zebrafish tail fin regeneration. Development 2014; 141: 2581–2591

[39] Brown BN, Sicari BM, Badylak SF. Rethinking regenerative medicine: Amacrophage-centered approach. Front Immunol 2014; 5: 510

[40] Pettitt D, Arshad Z, Davies B et al. An assessment of the factors affecting the commercialization of cell-based therapeutics: A systematic review protocol. Syst Rev 2017; 6: 120

[41] Brittberg M, Lindahl A, Nilsson A et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994; 331: 889–895

[42] Peterson L, Minas T, Brittberg M et al. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 2000, doi:212–234

[43] Lindahl A, Brittberg M, Peterson L. Health economics benefits following autologous chondrocyte transplantation for patients with focal chondral lesions of the knee. Knee Surg Sports Traumatol Arthrosc 2001; 9: 358–363

[44] Schuette HB, Kraeutler MJ, McCarty EC. Matrix-assisted autologous chondrocyte transplantation in the Knee: A systematic review of mid- to long-term clinical outcomes. Orthop J Sports Med 2017; 5: 2325967117709250

[45] Mumme M, Barbero A, Miot S et al. Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. Lancet 2016; 388: 1985–1994

[46] Pelttari K, Pippenger B, Mumme M et al. Adult human neural crest-derived cells for articular cartilage repair. Sci Transl Med 2014; 6: 251ra119

[47] Pelttari K, Mumme M, Barbero A et al. Nasal chondrocytes as a neural crest-derived cell source for regenerative medicine. Curr Opin Biotechnol 2017; 47: 1–6

[48] Fulco I, Miot S, Haug MD et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: An observational first-in-human trial. Lancet 2014; 384: 337–346

[49] Dodson BP, Levine AD. Challenges in the translation and commercialization of cell therapies. BMC Biotechnol 2015; 15: 70

[50] Stace ET, Dakin SG, Mouthuy PA et al. Translating Regenerative Biomaterials Into Clinical Practice. J Cell Physiol 2016; 231: 36–49

[51] McLaren A. Ethical and social considerations of stem cell research. Nature 2001; 414: 129–131

[52] Leeper NJ, Hunter AL, Cooke JP. Stem cell therapy for vascular regeneration: adult, embryonic, and induced pluripotent stem cells. Circulation 2010; 122: 517–526

[53] Frantz S. Embryonic stem cell pioneer Geron exits field, cuts losses. Nat Biotechnol 2012; 30: 12–13

[54] Kirouac DC, Zandstra PW. The systematic production of cells for cell therapies. Cell Stem Cell 2008; 3: 369–381

[55] Badylak S. Perspective: Work with, not against, biology. Nature 2016; 540: S55

[56] Yanaga H, Koga M, Imai K et al. Clinical application of biotechnically cultured autologous chondrocytes as novel graft material for nasal augmentation. Aesthetic Plast Surg 2004; 28: 212–221

[57] Yanaga H, Yanaga K, Imai K et al. Clinical application of cultured autologous human auricular chondrocytes with autologous serum for craniofacial or nasal augmentation and repair. Plast Reconstr Surg 2006; 117: 2019–2030 discussion 2031-2012

[58] Yanaga H, Imai K, Tanaka Y et al. Two-stage transplantation of cell-engineered autologous auricular chondrocytes to regenerate chondrofat composite tissue: Clinical application in regenerative surgery. Plast Reconstr Surg 2013; 132: 1467–1477

[59] Ceccarelli G, Gentile P, Marcarelli M et al. In vitro and in vivo studies of alar-nasal cartilage using autologous micro-grafts: the use of the Rigenera(R) protocol in the treatment of an osteochondral lesion of the nose. Pharmaceuticals (Basel) 2017; 10:

[60] Purpura V, Bondioli E, Graziano A et al. Tissue Characterization after a New Disaggregation Method for Skin Micro-Grafts Generation. J Vis Exp 2016, doi: doi:10.3791/53579 e53579

[61] Zhou L, Pomerantseva I, Bassett EK et al. Engineering ear constructs with a composite scaffold to maintain dimensions. Tissue Eng Part A 2011; 17: 1573–1581

[62] Pomerantseva I, Bichara DA, Tseng A et al. Ear-shaped stable auricular cartilage engineered from extensively expanded chondrocytes in an immunocompetent experimental animal model. Tissue Eng Part A 2016; 22: 197–207

[63] Cervantes TM, Bassett EK, Tseng A et al. Design of composite scaffolds and three-dimensional shape analysis for tissue-engineered ear. J R Soc Interface 2013; 10: 20130413

[64] Cao Y, Vacanti JP, Paige KT et al. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. Plast Reconstr Surg 1997; 100: 297–302 discussion 303-294

[65] Lee SJ, Broda C, Atala A et al. Engineered cartilage covered ear implants for auricular cartilage reconstruction. Biomacromolecules 2011; 12: 306–313

[66] Yanaga H, Imai K, Fujimoto T et al. Generating ears from cultured autologous auricular chondrocytes by using two-stage implantation in treatment of microtia. Plast Reconstr Surg 2009; 124: 817–825

[67] Yanaga H, Imai K, Koga M et al. Cell-engineered human elastic chondrocytes regenerate natural scaffold in vitro and neocartilage with neoperichondrium in the human body post-transplantation. Tissue Eng Part A 2012; 18: 2020–2029

[68] Firmin F. Ear reconstruction in cases of typical microtia. Personal experience based on 352 microtic ear corrections. Scand J Plast Reconstr Surg Hand Surg 1998; 32: 35–47

[69] Firmin F. State-of-the-art autogenous ear reconstruction in cases of microtia. Adv Otorhinolaryngol 2010; 68: 25–52

[70] Utomo L, Pleumeekers MM, Nimeskern L et al. Preparation and characterization of a decellularized cartilage scaffold for ear cartilage reconstruction. Biomed Mater 2015; 10: 015010

[71] Ozmen OA, Falcioni M, Lauda L et al. Outcomes of facial nerve grafting in 155 cases: Predictive value of history and preoperative function. Otol Neurotol 2011; 32: 1341–1346

[72] Ray WZ, Mackinnon SE. Management of nerve gaps: Autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. Exp Neurol 2010; 223: 77–85

[73] Gaudin R, Knipfer C, Henningsen A et al. Approaches to peripheral nerve Repair: Generations of biomaterial conduits yielding to replacing autologous nerve grafts in craniomaxillofacial surgery. Biomed Res Int 2016; 2016: 3856262

[74] Brunelli GA, Vigasio A, Brunelli GR. Different conduits in peripheral nerve surgery. Microsurgery 1994; 15: 176–178

[75] Navissano M, Malan F, Carnino R et al. Neurotube for facial nerve repair. Microsurgery 2005; 25: 268–271

[76] Jiang X, Lim SH, Mao HQ et al. Current applications and future perspectives of artificial nerve conduits. Exp Neurol 2010; 223: 86–101

[77] Inada Y, Hosoi H, Yamashita A et al. Regeneration of peripheral motor nerve gaps with a polyglycolic acid-collagen tube: technical case report. Neurosurgery 2007; 61: E1105–E1107 discussion E1107

[78] Yamanaka T, Hosoi H, Murai T et al. Regeneration of the nerves in the aerial cavity with an artificial nerve conduit – reconstruction of chorda tympani nerve gaps. PLoS One 2014; 9: e92258

[79] Gunn S, Cosetti M, Roland JT Jr.. Processed allograft: Novel use in facial nerve repair after resection of a rare racial nerve paraganglioma. Laryngoscope 2010; 120: (Suppl 4): S206

[80] Kusaba H, Terada-Nakaishi M, Wang W et al. Comparison of nerve regenerative efficacy between decellularized nerve graft and nonwoven chitosan conduit. Biomed Mater Eng 2016; 27: 75–85

[81] Wang W, Itoh S, Takakuda K. Comparative study of the efficacy of decellularization treatment of allogenic and xenogeneic nerves as nerve conduits. J Biomed Mater Res A 2016; 104: 445–454

[82] Wust S, Muller R, Hofmann S. 3D Bioprinting of complex channels-Effects of material, orientation, geometry, and cell embedding. J Biomed Mater Res A 2015; 103: 2558–2570

[83] Roy N, Merrill RM, Gray SD et al. Voice disorders in the general population: prevalence, risk factors, and occupational impact. Laryngoscope 2005; 115: 1988–1995

[84] Benninger MS, Alessi D, Archer S et al. Vocal fold scarring: current concepts and management. Otolaryngol Head Neck Surg 1996; 115: 474–482

[85] Li L, Stiadle JM, Lau HK et al. Tissue engineering-based therapeutic strategies for vocal fold repair and regeneration. Biomaterials 2016; 108: 91–110

[86] Fishman JM, Wiles K, Lowdell MW et al. Airway tissue engineering: an update. Expert Opin Biol Ther 2014; 14: 1477–1491

[87] Fishman JM, Long J, Gugatschka M et al. Stem cell approaches for vocal fold regeneration. Laryngoscope 2016; 126: 1865–1870

[88] Bartlett RS, Thibeault SL, Prestwich GD. Therapeutic potential of gel-based injectables for vocal fold regeneration. Biomed Mater 2012; 7: 024103

[89] Ling C, Li Q, Brown ME et al. Bioengineered vocal fold mucosa for voice restoration. Sci Transl Med 2015; 7: 314ra187

[90] Hung SH, Su CH, Lee FP et al. Larynx decellularization: combining freeze-drying and sonication as an effective method. J Voice 2013; 27: 289–294

[91] Baiguera S, Gonfiotti A, Jaus M et al. Development of bioengineered human larynx. Biomaterials 2011; 32: 4433–4442

[92] Hamilton NJI, Birchall MA. Tissue-Engineered Larynx: Future Applications in Laryngeal Cancer. Curr Otorhinolaryngol Rep 2017; 5: 42–48

[93] Farwell DG, Birchall MA, Macchiarini P et al. Laryngotracheal transplantation: technical modifications and functional outcomes. Laryngoscope 2013; 123: 2502–2508

[94] Krishnan G, Du C, Fishman JM et al. The current status of human laryngeal transplantation in 2017: A state of the field review. Laryngoscope 2017; 127: 1861–1868

[95] Zopf DA, Hollister SJ, Nelson ME et al. Bioresorbable airway splint created with a three-dimensional printer. N Engl J Med 2013; 368: 2043–2045

[96] Delaere PR, Van Raemdonck D. The trachea: The first tissue-engineered organ? J Thorac Cardiovasc Surg 2014; 147: 1128–1132

[97] Delaere P, Van Raemdonck D. Tracheal replacement. J Thorac Dis 2016; 8: S186–S196

[98] Macchiarini P, Jungebluth P, Go T et al. Clinical transplantation of a tissue-engineered airway. Lancet 2008; 372: 2023–2030

[99] Vogel G. Trachea transplants test the limits. Science 2013; 340: 266–268

[100] Cyranoski D. Surgeon commits misconduct. Nature 2015; 521: 406–407

[101] Hamilton NJ, Kanani M, Roebuck DJ et al. Tissue-engineered tracheal replacement in a child: A 4-year follow-up study. Am J Transplant 2015; 15: 2750–2757

[102] Elliott MJ, De Coppi P, Speggiorin S et al. Stem-cell-based, tissue engineered tracheal replacement in a child: A 2-year follow-up study. Lancet 2012; 380: 994–1000

[103] Villar-Fernandez MA, Lopez-Escamez JA. Outlook for tissue engineering of the tympanic membrane. Audiol Res 2015; 5: 117

[104] Kanemaru S, Umeda H, Kitani Y et al. Regenerative treatment for tympanic membrane perforation. Otol Neurotol 2011; 32: 1218–1223

[105] Jackler RK. A regenerative method of tympanic membrane repair could be the greatest advance in otology since the cochlear implant. Otol Neurotol 2012; 33: 289

[106] Omae K, Kanemaru SI, Nakatani E et al. Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor. Auris Nasus Larynx 2017; 44: 664–671

[107] Kanemaru S, Umeda H, Kanai R et al. Regenerative treatment for soft tissue defects of the external auditory meatus. Otol Neurotol 2014; 35: 442–448

[108] Kuo CY, Wilson E, Fuson A et al. Repair of tympanic membrane perforations with customized bioprinted ear grafts using chinchilla models. Tissue Eng Part A 2017, doi: doi:10.1089/ten.TEA.2017.0246

[109] Vos JD, Latev MD, Labadie RF et al. Use of AlloDerm in type I tympanoplasty: a comparison with native tissue grafts. Laryngoscope 2005; 115: 1599–1602

[110] Haynes DS, Vos JD, Labadie RF. Acellular allograft dermal matrix for tympanoplasty. Curr Opin Otolaryngol Head Neck Surg 2005; 13: 283–286

[111] Fishman AJ, Marrinan MS, Huang TC et al. Total tympanic membrane reconstruction: AlloDerm versus temporalis fascia. Otolaryngol Head Neck Surg 2005; 132: 906–915

[112] Kaftan H. [Tympanic membrane reconstruction with non-autogenous transplants and alloplastic materials]. Laryngorhinootologie 2010; 89: 562–568 quiz 569-570

[113] Schwarz D, Pazen D, Gosz K et al. Acoustic properties of collagenous matrices of xenogenic origin for tympanic membrane reconstruction. Otol Neurotol 2016; 37: 692–697

[114] Kanemaru S, Nakamura T, Omori K et al. Regeneration of mastoid air cells: Clinical applications. Acta Otolaryngol Suppl 2004, doi:80-84

[115] Kanemaru S, Nakamura T, Omori K et al. Regeneration of mastoid air cells in clinical applications by in situ tissue engineering. Laryngoscope 2005; 115: 253–258

[116] Sade J, Luntz M, Levy D. Middle ear gas composition and middle ear aeration. Ann Otol Rhinol Laryngol 1995; 104: 369–373

[117] Kanemaru S, Umeda H, Yamashita M et al. Improvement of eustachian tube function by tissue-engineered regeneration of mastoid air cells. Laryngoscope 2013; 123: 472–476

[118] Gould TW, Birchall JP, Mallick AS et al. Development of a porous poly(DL-lactic acid-co-glycolic acid)-based scaffold for mastoid air-cell regeneration. Laryngoscope 2013; 123: 3156–3161

[119] Jang CH, Cho YB, Kim JS et al. Regeneration of mastoid air cells using polycaprolactone/beta-tricalcium phosphate biocomposites: an Experimental study. Laryngoscope 2012; 122: 660–664

[120] Lyon J. Hearing Restoration: A Step Closer? JAMA 2017; 318: 319–320

[121] McLean WJ, Yin X, Lu L et al. Clonal Expansion of Lgr5-Positive Cells from Mammalian Cochlea and High-Purity Generation of Sensory Hair Cells. Cell Rep 2017; 18: 1917–1929

[122] Vergeer MR, Doornaert PA, Rietveld DH et al. Intensity-modulated radiotherapy reduces radiation-induced morbidity and improves health-related quality of life: results of a nonrandomized prospective study using a standardized follow-up program. Int J Radiat Oncol Biol Phys 2009; 74: 1–8

[123] Scott-Brown M, Miah A, Harrington K et al. Evidence-based review: quality of life following head and neck intensity-modulated radiotherapy. Radiother Oncol 2010; 97: 249–257

[124] Riley P, Glenny AM, Hua F et al. Pharmacological interventions for preventing dry mouth and salivary gland dysfunction following radiotherapy. Cochrane Database Syst Rev 2017; 7: CD012744

[125] van Luijk P, Pringle S, Deasy JO et al. Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer. Sci Transl Med 2015; 7: 305ra147

[126] Lombaert I, Movahednia MM, Adine C et al. Concise Review: Salivary Gland Regeneration: Therapeutic Approaches from. Stem Cells to Tissue Organoids. Stem Cells 2017; 35: 97–105

[127] Nagler RM. The enigmatic mechanism of irradiation-induced damage to the major salivary glands. Oral Dis 2002; 8: 141–146

[128] Nagler RM. Effects of head and neck radiotherapy on major salivary glands – animal studies and human implications. In Vivo 2003; 17: 369–375

[129] Redman RS. On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development. Biotech Histochem 2008; 83: 103–130

[130] Schwarz S, Huss R, Schulz-Siegmund M et al. Bone marrow-derived mesenchymal stem cells migrate to healthy and damaged salivary glands following stem cell infusion. Int J Oral Sci 2014; 6: 154–161

[131] Lim JY, Yi T, Choi JS et al. Intraglandular transplantation of bone marrow-derived clonal mesenchymal stem cells for amelioration of post-irradiation salivary gland damage. Oral Oncol 2013; 49: 136–143

[132] Lim JY, Ra JC, Shin IS et al. Systemic transplantation of human adipose tissue-derived mesenchymal stem cells for the regeneration of irradiation-induced salivary gland damage. PLoS One 2013; 8: e71167

[133] Sumita Y, Liu Y, Khalili S et al. Bone marrow-derived cells rescue salivary gland function in mice with head and neck irradiation. Int J Biochem Cell Biol 2011; 43: 80–87

[134] Pringle S, Nanduri LS, van der Zwaag M et al. Isolation of mouse salivary gland stem cells. J Vis Exp 2011, doi:10.3791/2484

[135] Pringle S, Maimets M, van der Zwaag M et al. Human Salivary Gland Stem Cells Functionally Restore Radiation Damaged Salivary Glands. Stem Cells 2016; 34: 640–652

[136] Nanduri LS, Maimets M, Pringle SA et al. Regeneration of irradiated salivary glands with stem cell marker expressing cells. Radiother Oncol 2011; 99: 367–372

[137] Lombaert IM, Brunsting JF, Wierenga PK et al. Rescue of salivary gland function after stem cell transplantation in irradiated glands. PLoS One 2008; 3: e2063

[138] Comella K, Bell W. First-in-man intraglandular implantation of stromal vascular fraction and adipose-derived stem cells plus platelet-rich plasma in irradiation-induced gland damage: a case study. Int Med Case Rep J 2017; 10: 295–299

[139] Gronhoj C, Jensen DH, Glovinski PV et al. First-in-man mesenchymal stem cells for radiation-induced xerostomia (MESRIX): study protocol for a randomized controlled trial. Trials 2017; 18: 108

[140] Ferreira JN, Rungarunlert S, Urkasemsin G et al. Three-Dimensional Bioprinting Nanotechnologies towards Clinical Application of Stem Cells and Their Secretome in Salivary Gland Regeneration. Stem Cells Int 2016; 2016: 7564689

[141] Flores RL, Liss H, Raffaelli S et al. The technique for 3D printing patient-specific models for auricular reconstruction. PlumX Metrics 2017; 6: 937–943

[142] Otto IA, Melchels FPM, Randolph MA et al. Auricular reconstruction using biofabrication-based tissue engineering strategies. Biofabrication 2015; 7 (3)