Review

Discovery Medicine 2025; 37(192): 19–30
https://doi.org/10.24976/Discov.Med.202537192.2

Salivary Glands: Function, Dysfunction, Regeneration, and Repair

Marco Tatullo[1,2,3,*], Gianfranco Favia[4], Nicola Antonio Adolfo Quaranta[1], Andrea Pacifici[5], Mohammad Islam[3,†], Pia Lopez-Jornet[6,†]

[1]Department of Translational Biomedicine and Neuroscience – DiBraiN, University of Bari "Aldo Moro", 70124 Bari, Italy
[2]MIRROR-Medical Institute for Regeneration and Repairing and Organ Replacement, Interdepartmental Center, University of Bari "Aldo Moro", 70124 Bari, Italy
[3]School of Dentistry, University of Dundee, DD1 4HN Dundee, UK
[4]Department of Interdisciplinary Medicine, University of Bari "Aldo Moro", 70124 Bari, Italy
[5]Department of Oral and Maxillo-Facial Sciences, Sapienza University of Rome, 00195 Rome, Italy
[6]Department of Stomatology, Faculty of Dentistry, University of Murcia, 30008 Murcia, Spain
*Correspondence: marco.tatullo@uniba.it (Marco Tatullo)
[†]These authors contributed equally.
Published: 20 January 2025

Salivary gland dysfunctions are common conditions variously related to aging, inflammatory players, and any other factor able to alter their normal physiology. These conditions may significantly impact oral and systemic health, affecting the overall quality of life. Over time, numerous therapeutic strategies have been explored to regenerate, repair, or replace injured salivary glands, focusing on those molecular and cellular mechanisms able to be safely translated into a clinical landscape. In this context, stem cells, tissue engineering, and the novel organoids technology, have gained exciting results, even if such approaches may require some optimization for their long-term maintenance. Despite extensive research, a composite stem cell population capable of regenerating functional glandular tissue remains elusive; nonetheless, to overcome these current limitations, recently, the transplantation of allogeneic stem cells has emerged as a reliable solution. This overview comprehensively examines the salivary glands in the light of modern biotechnologies, with the aim of better understanding the current state of the art in salivary gland regeneration and repair by using tissue engineering, biomimetic strategies, target therapies, and three-dimensional (3D) organoids technology. This work investigates the main salivary gland dysfunctions and their impact on oral and systemic health. It then discusses the most promising advanced strategies for oral tissue bioengineering, focusing on the potential of stem cells and organoids.

Keywords: salivary glands; oral medicine; regenerative medicine; tissue engineering; translational medicine

Introduction

Salivary glands (SG) play a crucial role in maintaining oral health, and their dysfunction can significantly affect oral function and overall health-related outcomes [1]. Currently, available therapeutic options for the treatment of hyposalivation focus primarily on palliative care that provides only temporary relief of symptoms, without addressing the underlying SG dysfunction [2]. This limitation highlights the urgent need for innovative strategies to restore SG function. In this context, a promising avenue is SG re-engineering, which could potentially lead to permanent restoration of salivary flow and improved quality of life for those affected [3]. Several research groups have investigated the potential benefits of combining biomaterials with various cell types to create an optimal environment for regenerating the structure and function of various tissues, including SG [3–5]. This multidisciplinary approach aims to replicate both the natural architecture and functions of SG to improve the effectiveness of the forthcoming regenerative therapies. Despite advancements in cell therapy and SG bioengineering, there are still significant gaps in our understanding of the complex mechanisms that govern SG development, function, and repair. To address these gaps, this review offers a comprehensive examination of human major SG anatomy, structure, function, and associated dysfunctions. Additionally, we explore critical co-factors in SG bioengineering, proposing promising strategies, discussing current challenges, and outlining future directions for the development of effective therapeutic interventions. This comprehensive approach aims to contribute to the advancement of regenerative therapies for SG dysfunctions.

Salivary Glands Dysfunction: Challenges and Solution

Overview of Salivary Glands: Structure and Function

SG exhibits a complex and diverse anatomical structure, and its dysfunction can lead to a wide range of pathologies. SG are essentially exocrine secretory structures derived from the epithelial lining of the upper aerodigestive tract [4]. Their primary physiological function is the secretion of saliva, a vital fluid that plays significant roles in lubrication, digestion, immunological function, and the overall maintenance of both oral and systemic health. Extensive studies have elucidated the anatomy and histology of SG [2,4], revealing their organization as branched organs characterized by a highly intricate ductal system. This ductal network culminates in secretory units known as acini, which comprise serous and mucous acinar cells; each cell type uniquely contributes to the composition of saliva [5]. Moreover, SG are densely innervated by the autonomic nervous system, highlighting their essential role in regulating salivary secretion.

SG can be classified into two main categories: major and minor glands. The terms "major" and "minor" refer to the anatomical size of the glands and the amount of saliva they produce. In detail, the major SG are bilaterally paired structures categorized into three distinct types: the parotid gland (PG); submandibular gland (SMG); and sublingual gland (SLG). These major glands consist of poly-lobular aggregates enclosed in a thin fibrous capsule, allowing for efficient secretion and drainage of saliva. In addition to these, hundreds of minor SG are distributed throughout the oral cavity (OC), particularly in the labial and lingual mucosa, as well as the palate and floor of the mouth [5].

Focusing on the major SG, their anatomical architecture reveals remarkable similarities. The PG, for instance, is predominantly composed of serous acini that secrete saliva rich in α-amylase, an enzyme crucial for carbohydrate digestion. In contrast, the SLG produced a mucous secretion, a viscous solution rich in mucins. The SMG, on the other hand, contains a mixture of both mucous and serous acini, allowing it to produce a more balanced saliva composition [6]. The average daily salivary volume secreted by these glands ranges from approximately 1.0 to 1.5 litres; however, this production is not constant. Salivary flow varies quantitatively: it is lowest during sleep, increases during speech, and peaks during meals. These fluctuations are intricately regulated by the autonomic nervous system, particularly its parasympathetic component, which stimulates salivary secretion in response to various stimuli. Additionally, salivary secretion is heterogeneous, with significant variability depending on the specific gland and its anatomical location. Notably, in humans, over 90% of the total daily saliva production is attributed to the three major pairs of SG, while the remaining 10% is secreted by the numerous minor SG dispersed throughout the OC [4].

The Impact of SG Dysfunction on Oral and Systemic Health

SG dysfunction can lead to both quantitative and qualitative alterations in salivary composition and flow, resulting in conditions such as hyposalivation (manifesting as "xerostomia", often perceived by the patient as a "dry mouth" sensation) or hypersalivation (sialorrhea or hypersialia) [7,8]. These changes can significantly affect the patient's quality of life, leading to direct functional impairments and an increased susceptibility to various pathological conditions [8]. The aetiology of SG dysfunction is multifactorial, encompassing autoimmune disorders, cancer, infections, and the effects of radiation therapy (RT) [7,8]. A prominent example is Sjogren's syndrome, an autoimmune disorder characterized by exocrine gland dysfunction, which can severely impact a patient's quality of life through debilitating xerostomia.

SG hypofunction exacerbates both oral and systemic diseases, disrupts the oral microbiome, and adversely impacts digestion [8]. Additionally, certain pharmaceutical agents can induce SG dysfunction by influencing salivary flow rates and composition. Drugs targeting the nervous, cardiovascular, genitourinary, musculoskeletal, respiratory, and digestive systems are particularly associated with these effects [9]. Furthermore, SG dysfunction and irreversible hyposalivation are significant side effects of RT treatment for head and neck cancer (HNC) [8].

In response to these challenges, several research groups are actively investigating methods to repair or regenerate SG, exploring innovative and promising strategies for the near future. In this perspective, regenerative medicine holds the potential to replace or regenerate exocrine organs such as the SG, thereby restoring their essential functions. A major goal of these regenerative strategies is to enhance the secretory function of damaged SG and increase saliva production. Given the limitations of current treatments (e.g., stimulant medications, salivary substitutes, and artificial saliva), three promising avenues for advancing regenerative therapies have been identified: (i) gene therapy; (ii) cell therapy; and (iii) bioengineered SG models [10], as illustrated in Fig. 1.

The Complexity of Oncological Aspects in the Salivary Glands

HNC encompasses a diverse group of malignant neoplasms that can arise in various locations within the upper aerodigestive tract, including the SG, nasopharynx, nasal cavities, and paranasal sinuses. SG tumours represent a particularly heterogeneous subset of these neoplasms, characterized by their rarity, with an incidence of 0.6 to 1.4 per 100,000 individuals [11]. These tumours account

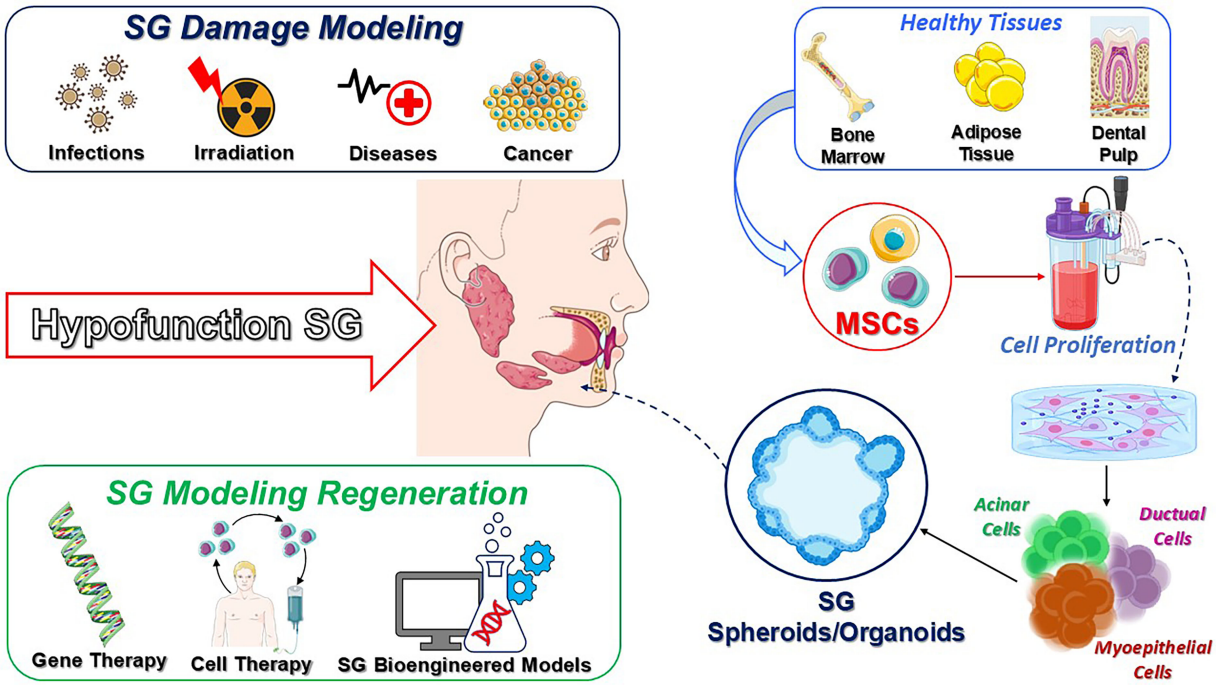

Fig. 1. Potential therapeutic approaches for salivary glands (SG)'s dysfunction. The use of gene therapy, cell therapy and bio-engineering has improved the potentialities to obtain replacing of healthy tissues. The SG spheroids or organoids are strategic tools for future personalized therapies. MSCs, mesenchymal stem cells (figure drawn with Microsoft-Powerpoint 2021, Microsoft Corporation, Redmond, WA, USA).

for approximately 1% of all neoplasms and 3% to 6% of all HNCs, predominantly affecting the major SG: the PG (75%), the SMG (7% to 11%), and the SLG (1%) [12] with a slight male predominance [6]. The development of SG neoplasms is multifactorial, involving complex interactions between genetic and environmental factors. Autoimmune disorders, viral infections, obesity, and chemical exposure have also been implicated in their aetiology [13]. Additionally, SG can harbour metastatic cells from other primary cancers, particularly skin cancers [6].

SG are highly differentiated and slowly proliferating tissues; however, they are particularly susceptible to the effects of RT. It is estimated that over 70% of patients with HNC who undergo RT will experience xerostomia and SG's dysfunction [14]. The adverse effects of RT on SG are both immediate and long-lasting, occurring during or shortly after treatment [15]. RT-induced SG dysfunction includes hyposalivation, xerostomia, oral infections, and functional impairments such as dysphagia, difficulties in chewing and swallowing, loss of taste, and also dental problems [16]. Notably, salivary secretion declines rapidly in the first week after RT, followed by a significant decrease over the next three months. Other aspects of saliva, such as salivary electrolyte levels, antibacterial capabilities, and buffering capacity, are also adversely affected. For instance, the pH of saliva decreases from 7.0 to 5.0, while concentrations of inorganic salts (such as calcium, sodium, and chloride) and organic molecules (including lysozyme) increase [17].

Intriguingly, ionizing radiation (IR) is a recognized risk factor for SG damage, particularly affecting the PG, during both RT and radiological examinations [18]. While IR-induced SG damage is clinically significant, the precise mechanisms underlying this damage remain not completely understood [19]. The side effects of IR are both time- and dose-dependent, with parenchymal damage being particularly severe when tissues are directly exposed to the radiation field [20]. A prominent consequence of late irradiation-induced SG damage is the development of chronic inflammation and fibrosis, leading to tissue dysfunction and atrophy. Evidence suggests that serous acinar cells are more radiosensitive than mucous acinar cells, indicating that PG, consisting entirely of serous acinar cells, may be more susceptible to radiation damage than SMG or SLG [20].

Significant advancements have been made in the field of RT, primarily aimed at minimizing damage to surrounding tissues. This has been achieved through modified treatment protocols, technological enhancements, and the development of new radiation sources. In this context, intensity-modulated radiotherapy (IMRT) is considered a more effective strategy than conventional RT. IMRT can reduce the severity of SG hypofunction and xerostomia while enabling more precise delivery of radiation doses and providing greater protection to surrounding healthy tissues [21].

Advanced Strategies for Oral Tissue Bioengineering: Focus on Stem Cells and Engineered Organoids

Main Approaches in Oral Regeneration and Repair

Oral tissue bioengineering has emerged as a promising alternative to conventional biomaterials for the reconstruction of tissues and organs in the oral and craniofacial region. However, it faces limitations related to the body's natural regenerative capacity and the current materials and methods available [22]. Functional SG development requires complex interactions between ductal, acinar and myoepithelial cells within a branched structure. Recent technological advancements have facilitated the exploration of molecular and cellular mechanisms underlying SG function [18]. Notably, three-dimensional (3D) cell cultures, which mimic the architecture of glandular organs, can replicate SG behaviour under both physiological and pathological conditions [23].

In this context, tissue engineering is driven by the need to repair and regenerate various damaged tissues (e.g., epithelial, vascular, nerve, bone, cartilage, glandular, etc.) in patients suffering from severe trauma or injury. Ongoing scientific research aims to optimize conditions for aesthetic and functional rehabilitation through cell cluster implants or whole organ replacement [24,25]. Recent studies have demonstrated the potential of tissue engineering for SG regeneration [23]. Both preclinical and clinical investigations have highlighted the significant role of mesenchymal stem cells (MSCs) in tissue homeostasis and their promising applications in tissue engineering and regenerative medicine [24,25]. MSCs are particularly attractive candidates for treating various pathologies due to their ability to modulate inflammation, enhance angiogenesis, reduce tissue fibrosis, and mediate immunosuppressive effects [26]. Furthermore, their multipotential differentiation capacity, self-renewal ability, and migratory properties make them highly suitable for clinical applications [25,27].

Clinical Insights and Translational Impact of SG Regeneration

Major clinical trials investigating stem cell therapies for SG dysfunction are summarized in Table 1 (Ref. [18, 26,28–31]). Despite the slow turnover of SG tissue, several studies on SG injury have revealed significant regenerative potential, varying depending on the nature and extent of the damage. Moreover, a complex network of molecular pathways is required for the full functionality of SG [32].

Given this complexity, numerous molecular signalling pathways have been studied, some of which are discussed below and summarized in Table 2 (Ref. [33–41]). Notably, previous research has highlighted the expression of neurofibromin, a protein encoded by the *NF1* gene, in the ducts of SG [33]. Scientific evidence suggests that alterations in neurofibromin, resulting from *NF1* gene mutations, may impair SG function. In light of these findings, researchers investigated neurofibromin expression in acinar and ductal cells of both major and minor SG in individuals without neurofibromatosis 1 (NF1) [34]. The results revealed that neurofibromin was expressed in both serous and mucous acinar cells, with higher expression in ductal cells. However, the precise role of neurofibromin in adult human SG development, morphology, and functionality remains unclear, necessitating further research.

Additionally, inorganic nitrate, abundant in plant foods, has been shown to mitigate damage caused by reactive oxygen species (ROS)-induced oxidative stress. Research suggests that nitrate inhibits the Nod-Like Receptor Protein 3 (NLPR3) inflammasome-mediated pyroptosis and reduces ROS levels, thereby maintaining mitochondrial homeostasis. Furthermore, inorganic nitrate may promote SG cell regeneration and reduce apoptosis by increasing Sialin levels [35] (Fig. 2).

Currently, two primary methods are employed for delivering cells for SG regeneration: systemic intravenous injection and percutaneous direct injection into the SG with ultrasound guidance. While effective, these methods have complications, including potential side effects in other organs and an increased risk of infection. To address these issues, catheter-based cell delivery has been proposed [42]. However, the risk of uncontrolled proliferation of transplanted cells poses a significant concern for carcinogenesis. Therefore, researchers have increasingly focused on paracrine factors, such as conditioned media, secretomes, and exosomes [43].

Interestingly, numerous studies have highlighted the potential of SG stem/progenitor cells as a novel therapeutic strategy for treating SG dysfunction [16]. Recent approaches emphasize the use of cell lines instead of primary cells, yielding promising preliminary results. Nevertheless, no existing cell line fully recapitulates the morphology and functionality of SG [6]. Advancements in 3D cell culture biotechnologies have recently led to the development of customized techniques that could represent a reliable and translational strategy to repair SG; these techniques include the formation of:

(i) spheroids, which consist of aggregations of cells derived from a single cell or a mixture of cells [44];

(ii) organoids, which are generated three-dimensionally in plates from single stem cells (including tissue-specific stem cells and pluripotent stem cells) and retain physiological functions similar to those of the original organs or tissues [45];

and, (iii) tumoroids, which can be described as "tumour-like organoids", typically derived from primary tumours [46]. The use of spheroids has demonstrated efficacy in overcoming the limitations inherent to conventional *in vitro* cell culture methodologies. In particular, spheroids can more accurately mimic solid tumours, thereby providing detailed insights into the tumour microenvironment

Table 1. Major clinical trials investigating stem cell therapies in SG's dysfunction.

NCT number	Cell type	Transplantation source	Delivery method	Outcomes	Recruitment status	Start date	References
NCT02513238	HAd-MSCs	Autologous	Intraglandular	Safety and efficacy	Completed	2015	[30]
NCT03743155	BM-MSCs	Autologous	Intraglandular	Safety and efficacy	Completed	2018	[18]
NCT03874572	ADSCs	Allogeneic	Intraglandular	Safety and efficacy	Active, not recruiting	2019	[29]
NCT04007081	BM-MSCs	Autologous	Intraglandular	Safety and efficacy	Completed	2019	[28]
NCT04593589	SG-MSCs	Autologous	Intraglandular	Safety and feasibility	Recruiting	2022	[26]
NCT05820711	BM-MSCs	Autologous	Injection into submandibular glands	Safety and feasibility	Recruiting	2023	[31]

HAd-MSCs, human adipose tissue-derived mesenchymal stem cells; BM-MSCs, Bone marrow-derived mesenchymal stem cells; ADSCs, adipose-derived mesenchymal stem cells; SG-MSCs, salivary gland mesenchymal stem cells; NCT, National Clinical Trial.

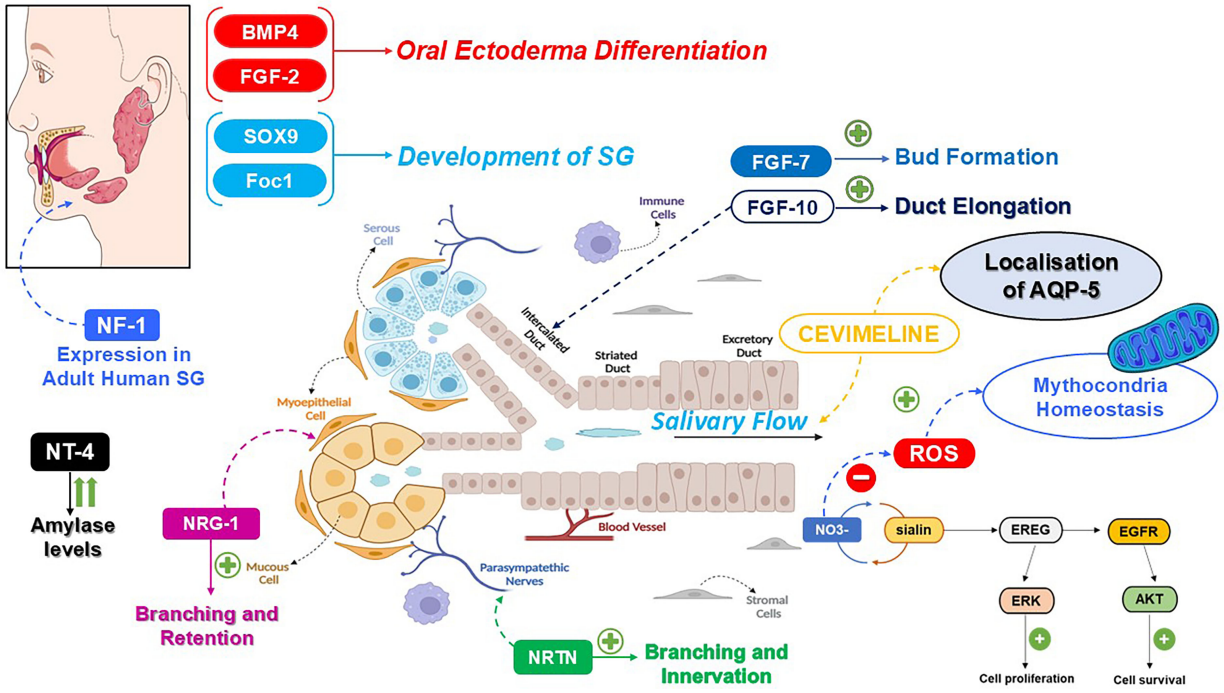

Fig. 2. Network of molecular signalling pathways required for the functionality of SG. The figure emphasizes the intricate interplay of various factors and signalling pathways in salivary gland development, function, and regeneration. The top left corner focuses on factors crucial for SG development. Bone morphogenetic protein 4 (BMP4) and Fibroblast growth factor 2 (FGF-2): induce oral ectoderm differentiation, the precursor to SG tissue. SRY-box transcription factor 9 (SOX9) and Forkhead box protein C1 (Foc1): essential transcription factors for SG development. FGF-7 and FGF-10: promote branching morphogenesis, guiding the formation of buds (FGF-7) and ductal elongation (FGF-10). The central part of the figure highlights pathways involved in SG function and regeneration. Neurofibromatosis 1 (NF1): a protein expressed in adult human SG, potentially influencing its function. Neurotrophin 4 (NT-4): increases amylase levels, a key enzyme in saliva. Neuregulin 1 (NRG-1): promotes branching and retention of acinar cells. Neurturin (NRTN): induces branching and innervation of the SG. Cevimeline: a drug that influences Aquaporin 5 (AQP-5) localization (water channels), salivary flow, and potentially regeneration. The right side of the figure focuses on cellular processes and signaling. Reactive oxygen species (ROS): they are often implicated in cell damage. Nitrate (NO3-): can mitigate ROS-induced damage, promoting mitochondrial homeostasis. Sialin: involved in SG cell regeneration and apoptosis reduction. Epiregulin (EREG) and Epidermal growth factor receptor (EGFR): epiregulin and its receptor, are involved in cell proliferation and survival through the Extracellular signal-regulated kinase (ERK) and Protein kinase B (AKT) pathways (figure drawn with Microsoft-Powerpoint 2021, Microsoft Corporation, Redmond, WA, USA).

(TME); nonetheless, spheroids still face limitations regarding self-assembly and regeneration [47].

Importantly, stem cell and organoid based regenerative therapies for salivary glands hold great promise but

Table 2. Major clinical trials investigating stem cell therapies in SG's dysfunction.

Markers	Action	References
Cevimeline	- Regeneration of innervated grape - Maintenance of salivary secretion (via M3 muscarinic receptors) - Influence on the localisation of AQP-5	[36]
NF1	- Potential expression in adult human SG (acini e ducts)	[33,34]
NT-4	- Increases amylase levels	[37]
NRTN	- Initiates branching and innervation	[38]
NRG-1	- Promotes branching and retention of acinar-like cells	[39]
BMP4, FGF-2	- Oral ectodermal differentiation	[40]
SOX9, Foc1, FGF-10, FGF-7	- Essential for the development of these structures - FGF-7 promotes trailing bud formation and FGF-10 promotes duct elongation	[41]
Inorganic nitrate	- Inhibits NLPR3 inflammasome-mediated pyroptosis - Reduces ROS levels to maintain mitochondrial homeostasis - Promotes salivary gland cell regeneration and reduces apoptosis	[35]

NLPR3, Nod-Like Receptor Protein 3.

also come with potential risks and complications; one such concern is the risk of tumour formation due to uncontrolled proliferation of transplanted stem cells or cells within organoids which necessitates rigorous characterization and purification of cell populations. Moreover, immune rejection is another challenge, particularly with allogeneic transplantation where cells or organoids from a donor are used, which might require immunosuppressive therapies with their own potential side effects; on the other hand, autologous transplantation using the patient's own cells can minimize this risk [36,44–47].

Advanced Strategies for Regenerating SG after Tissue Damage

Currently, only a few studies have directly investigated the regeneration of damaged glandular acinar cells. Several regenerative strategies strive to preserve the existing acinar cells through the temporary inhibition of cell apoptosis and senescence, and restoring damaged tissues with cell-based approaches. Undoubtedly, the main interest of SG regeneration is the restoration of glandular functions, reduced or lost because of different types of tissue damage [21]. Interestingly, some muscarinic receptor agonists, such as cevimeline and pilocarpine, have been used to treat hyposalivation [48]. A recent study demonstrated that prolonged local administration of cevimeline can effectively treat SG damage following ionizing radiations (IR). It was, in fact, demonstrated that mimicking a prolonged cholinergic muscarinic input, through the administration of a hydrogel formulation of cevimeline and alginate, may facilitate the regeneration of innervated acini, also ensuring physiological salivary secretion levels, comparable to those observed in non-irradiated glands for more than 3 months [36]. Aquaporins (AQP) are a family of small, integral membrane proteins that act as channels for water and, in some cases, small solutes, to pass through cell membranes; the cevimeline seems to influence the salivary composition through a slight regulatory effect on AQP-5 localisation, also recruiting AQP-1 to rapidly enhance salivary secretion and function [49].

Developing innovative strategies to restore SG function in HNC patients is a major clinical challenge [21]. Recent research has focused on a deeper understanding of the molecular and cellular mechanisms underlying the SG cancer development, so as to better customize innovative *in vitro* study models, such as "*organ-on-chip*" and organoids, which can be considered as the forefront of biomedical research [6]. In this scenario, the regenerative potential of stem cell-based therapy has been extensively investigated, demonstrating the safety and efficacy of MSC therapy for SG dysfunction in HNC patients, especially those patients undergoing RT [50]. Scientific evidence has demonstrated that transplanting allogeneic SG-stem cells can restore SG function in irradiated mice [49], suggesting the enormous potential benefits for several clinical issues, such as xerostomia. However, a definitive stem cell population capable of regenerating the entire complex structure of SG remains unclear [28]; in fact, substantial gaps still persist between preclinical studies and the clinical application of MSC-based therapies for SG damage repair. Addressing these challenges is crucial for translating research findings into effective treatments [29]. Engineered organoids could be able to bridge the gap between *in vitro* and *in vivo* models [51–54]. Recently, human organoids have been utilized to create customized models of several human diseases [55], exhibiting organ-like architecture and functionality *in vivo* [56,57], thus making them a useful tool in diagnostics, drug screening [58,59], and personalised medicine [60,61].

The development of organoids is primarily dependent on the self-organization of various stem cell types, which are regulated by fundamental signaling pathways. Stem cells are essential for maintaining tissue homeostasis, and replacing damaged cells, thus restoring the functionality of

injured tissues. The use of adult stem cells (ASCs) [62], or human pluripotent stem cells (PSCs) [63], are safely used for *in vitro* regenerative studies on organoid models; nonetheless, adipose-derived mesenchymal stem cells (ADSCs) have recently demonstrated success and safety in phase I/II clinical studies on damaged SG, reporting an increased salivary flow from 30 to 50% in 1 to 4 months following treatment with autologous or allogeneic ADSCs [30]. Building on previous studies, dental pulp stem cells (DPSCs) may offer promising avenues for innovative approaches to SG repair [64]. Unfortunately, the development of SG organoids holds issues regarding our understanding of the behaviour of SG-derived cells, following the regenerative stimuli [65]. These limitations, however, should not be considered insurmountable, given the considerable advantages in using organoid platforms, which can reduce animal testing and enable large-scale drug toxicity screening and functional testing, paving the way for new discoveries in biomedical research [66].

Up to date, the two principal approaches for creating *in vitro* SG organoids have been the following:

(i) incubating SG-derived stem/progenitor cells in a 3D culture system to replicate the gland's structure by mimicking the *in vivo* regenerative processes [67]; and

(ii) inducing PSCs to generate embryonic SG, thereby simulating the physiological developmental process [68].

Scaffolds play a crucial role in tissue engineering, acting as extracellular matrix (ECM) to which cells can adhere, proliferate, and differentiate. However, current materials used in scaffold design often exhibit inadequate mechanical stiffness and morphology, complicating organoid generation. In this context, the most used scaffold, able to ensure cell culturing in a clinical-grade environment, is Matrigel, a well-known biomimetic, biocompatible, bioactive, and biodegradable scaffold, widely used for generating organoids able to reproduce both hard [69] and soft oral tissues [70–73].

Table 3 (Ref. [74–78]) presents a comprehensive overview of studies investigating the potential of human stem cell transplantation for SG regeneration, both *in vivo* and *ex vivo*, following tissue damage. More in detail, RT is a quite common therapeutic approach in head and neck cancer, often resulting in damage to salivary glands, leading to xerostomia severely impacting quality of life. Sumita *et al*. [74] demonstrated that bone marrow-derived cells improved salivary flow in irradiated mice. This suggests the potential of these cells to be used in generating salivary gland organoids for transplantation. Lim *et al*. [75] reported similar findings using human adipose-derived mesenchymal stem cells in a rat model. The accessibility and ease of isolation of these cells make them attractive candidates for organoid development. Importantly, Pringle *et al*. [76] provided evidence that human salivary gland stem cells can functionally restore damaged glands. Organoids derived from these cells could, thus, provide a patient-specific approach to salivary gland regeneration. Elsaadany *et al*. [77] further confirmed the therapeutic potential of bone marrow-derived mesenchymal stem cells in a rat model, supporting their potential for organoid generation and application. Finally, Al-Serwi *et al*. [78] showed that human dental pulp stem cells mitigated salivary gland injury in a rat model of diabetes. This highlights the potential of these readily accessible cells to be used for developing salivary gland organoids.

These findings underscore the potential of different stem cell populations to regenerate salivary gland function after SG tissue damage, paving the way for future clinical applications using organoid technology to improve the quality of life for head and neck cancer survivors.

According to the existing literature on SG regeneration, the selection of materials for *in vitro* 3D organoid growth remains a topic of considerable debate. Extensive scientific evidence indicates that the exclusive use of natural materials to reproduce biomimetic environments presents several drawbacks, including (i) inadequate mechanical performance and (ii) limited resistance to fluctuations in physical conditions (pH and temperature) [79]. These limitations have prompted the development of scaffolds incorporating synthetic and biocompatible polymeric materials, such as Poly Lactic Acid (PLA), Poly Capro-Lactone Acid (PLC) and Poly Lactic-co-Glycolic Acid (PLGA) [80,81]. Unfortunately, also these synthetic options exhibit uncontrolled degradation rates and a lack of bioactive sites, which restrict their application in organoid generation [82]. The third-generation biomaterials used in scaffolds for organoid production must be bioactive, biomimetic, and selectively resorbable [83]. A modified, biomimetic surface can serve as an artificial ECM, offering appropriate biological stimuli to facilitate tissue repair and regeneration [84]. Biomimetic scaffolds designed to support SG tissue regeneration are complex materials with multifunctional properties; the design of optimal scaffolds is commonly influenced by the manufacturing methods used: additive manufacturing (AM) is the ideal technique, which allows for the transformation of 3D digital models into solid clinical models through layer-by-layer printing [85]. Within this context, bioprinting has emerged as a promising AM technique for the generation of SG organoids. Adine *et al.* [86] developed a magnetic 3D bioprinting platform, wherein the surface membrane of human DPSCs (HDPSCs) was labeled with magnetic nanoparticles and subsequently seeded in 96-well plates to facilitate cell aggregation. Following incubation, the 3D spheroids grew up at obtaining 3D SG organoids with enhanced neuronal compartments and excellent secretory functions [86]. This model may be particularly useful for studying cell adhesion and the molecular and mechanical changes occurring within a precisely regulated environment. Future studies could be directed towards some novel techniques, like microfluidics, to accelerate the advancement of biomaterials for regenerative ap-

Table 3. Human-derived stem cell transplantation therapies for *in vivo* and *ex vivo* SG regeneration after tissue damages.

Cell type	Mode of administration	Functional outcomes	References
BMDCS	Intravenous injections of cells (2×/week for 6 weeks) in female mouse SG after IR	- Enhanced salivary flow rate - Differentiate into SG epithelial cells	[74]
HAd-MSCs	Intravenous injections of cells (1×/week for 3 weeks) in mouse SG after IR	- Enhanced salivary flow rate (∼2 times greater) - Differentiate into SG epithelial cells	[75]
HSGSCs	Intraglandular transplantation of cells (single dose) in mice 1 month after IR	- Normal SG morphology was replaced - Secretory function rescue (∼64%)	[76]
BM-MSCs	Intraglandular transplantation of cells (single dose) in albino rats immediately after IR	- Preserving SG morphology and function - Minimizing the side effects of radiation	[77]
HDPSCs	Intravenous transplantation on parotid gland injury in rat model of streptozocin (STZ)-induced type I diabetes	- Decreased blood glucose - Improved parotid gland weight - Differentiate into SG acinar, ductal, and myoepithelial cells - Enhanced salivary flow rate	[78]

IR, ionising radiation; BMDCs, Bone marrow-derived cells; HSGSCs, human salivary gland stem/progenitor cells; BM-MSCs, Bone marrow-derived mesenchymal stem cells; HDPSCs, human dental pulp stem cells.

plications, as well as for creating high-precision miniaturized bioreactors for a smart production of mini-organoids [86,87].

Discussion

The construction of engineered oral and maxillofacial organoids is gaining significant traction and is poised to lead to breakthroughs in global healthcare and dentistry. Regenerative medicine has attracted the attention and commitment of scientists and medical engineers for decades, to replace or guide the regeneration of damaged tissues. Although organoid technology has emerged recently, it requires further optimization for long-term maintenance. Notably, SG organoids have demonstrated considerable potential as a radical solution for xerostomia and have also exhibited the capacity to restore the functionality of radiation-damaged glands [28].

Advances in genetic manipulation have facilitated the creation of more sophisticated oral organoids. Gene editing techniques, particularly the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) system, have transformed biomedical and oncological research, enabling precise, efficient, and cost-effective genome modifications. A key advantage of this system is its ability to correct defective genes "*in situ*", maintaining physiological regulation and ensuring normal expression. Despite its recent emergence and rapid evolution, CRISPR/Cas9 has already demonstrated utility in both theoretical and applied research, with applications ranging from rare genetic diseases to cancer [88]. Recent studies have also investigated the potential applications of optogenetics in various fields, including ophthalmology, bone repair, heart failure recovery, stroke rehabilitation, tissue engineering and regenerative medicine. This innovative approach could facilitate the precise regulation of the spatial-temporal self-organization of stem cells [89].

While promising results have been achieved in repairing and regenerating damaged SG, future research should focus on optimizing organoid size and functionality [90]. Modulating cultures may be beneficial for ensuring glandular and cellular diversity of the SG. Previous studies have highlighted the importance of several neurotrophic factors, such as: (i) Neuregulin-1, which promotes stem cell differentiation and prevents single-cell dissociation, thereby facilitating the development of organoids with stable cell diversity [90]; (ii) Neurotrophin-4 (NT-4), which increases amylase levels [37]; and (iii) Neurturin (NRTN) which induces branching and innervation of the SG [38].

More recently, human "*organs-on-a-chip*", also known as micro-physiological systems, have emerged as a potential new approach to accurately replicate the physiology of organs and tissues, surpassing traditional two-dimensional (2D) cultures and animal models. Derived from human cells, these models exhibit human-like metabolic processes and cellular turnover. A growing number of research groups have successfully constructed a variety of human organs on microfluidic chips, including the liver, lung, intestine, blood vessels, heart, and multi-organ chips. These encouraging results suggest that SG chips may be developed in the near future. However, numerous technological challenges remain to be addressed to fully meet the needs of the research community. While these models present a significant opportunity, it is crucial to consider the ethical issues that may arise from their clinical use [31,91].

Conclusions

SG play a pivotal role in maintaining oral homeostasis. Understanding these glands and their functions can enhance our comprehension of the underlying mechanisms of various diseases, including cancer, inflammation, and wound healing. Recently, several innovative technological approaches have been developed to investigate the cel-

lular and molecular dynamics that govern homeostasis and maintenance within the oral cavity, particularly concerning SG structure and function. Promising therapeutic strategies for regenerating damaged SG include cell therapies, secretomes, gene therapies, and 3D organoids. Importantly, SG organoids can provide a distinctive platform for investigating the development, biology, and pathogenesis of these glands; in fact, the novel organoids are exceptionally biomimetic, effectively recapitulating the SG function and structure of *in vitro*. Nonetheless, further molecular and functional characterization of SG tissues is essential to develop more accurate regenerative procedures.

The concepts of tissue regeneration, repair, and replacing represent a spectrum of various approaches in addressing salivary gland dysfunction and degeneration. In this review, the SG regeneration has been summarized, describing the most effective approaches to generate and differentiate new salivary gland components; additionally, three-dimensional organoids, mimicking the natural architecture of salivary glands, are also crucial in promoting SG regeneration by providing a scaffold for cell growth and differentiation gene therapy and the use of growth factors like Neuregulin-1, Neurotrophin-4, and Neurturin further contributes to the regenerative process, by influencing cell behaviour and tissue development.

On the other hand, the SG dysfunction often leads to tissue degeneration that needs to be opportunely repaired. SG repair often involves mitigating the effects of injury or disease and promoting the self-healing mechanisms of the salivary glands: drug therapy using muscarinic receptor agonists like cevimeline can stimulate salivary secretion and potentially repair damaged tissue inorganic nitrate with its ability to reduce oxidative stress and promote cell regeneration also plays a role in the repair process.

Importantly, these concepts are not mutually exclusive and can be employed in combination to achieve optimal outcomes. Current research is actively exploring these avenues to develop effective treatments for salivary gland dysfunction with the ultimate goal of improving quality of life for patients suffering from conditions like xerostomia or SG carcinoma.

Looking ahead, the integration of microfluidic systems and the application of biomaterials could significantly enhance the physiological relevance of SG organoids, making them more suitable for biomedical applications. Modern biotechnologies hold great possibilities in future advancing of research and clinical procedures: organoids are the main platform where to develop the most impacting studies, taking advantage from their high biomimetics and their perfect transferability from bench to bedside.

Availability of Data and Materials

Not applicable.

Author Contributions

MT, GF, MI, and PLJ collected and analyzed the literature. NAAQ and AP provided help and advice on the structure and content of the paper. All authors were involved in the drafting and critical revision of the manuscript. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Almansoori AA, Kim B, Lee JH, Tran SD. Tissue Engineering of Oral Mucosa and Salivary Gland: Disease Modeling and Clinical Applications. Micromachines. 2020; 11: 1066.

[2] Parisis D, Chivasso C, Perret J, Soyfoo MS, Delporte C. Current State of Knowledge on Primary Sjögren's Syndrome, an Autoimmune Exocrinopathy. Journal of Clinical Medicine. 2020; 9: 2299.

[3] Hajiabbas M, D'Agostino C, Simińska-Stanny J, Tran SD, Shavandi A, Delporte C. Bioengineering in salivary gland regeneration. Journal of Biomedical Science. 2022; 29: 35.

[4] Chibly AM, Aure MH, Patel VN, Hoffman MP. Salivary gland function, development, and regeneration. Physiological Reviews. 2022; 102: 1495–1552.

[5] Piraino LR, Benoit DSW, DeLouise LA. Salivary Gland Tissue Engineering Approaches: State of the Art and Future Directions. Cells. 2021; 10: 1723.

[6] Porcheri C, Mitsiadis TA. Physiology, Pathology and Regeneration of Salivary Glands. Cells. 2019; 8: 976.

[7] Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. Journal of Oral Rehabilitation. 2018; 45: 730–746.

[8] Rocchi C, Emmerson E. Mouth-Watering Results: Clinical Need, Current Approaches, and Future Directions for Salivary Gland Regeneration. Trends in Molecular Medicine. 2020; 26: 649–669.

[9] Saito I. Pathology of salivary gland dysfunction and restoration of function. Pathology International. 2021; 71: 304–315.

[10] Klangprapan J, Souza GR, Ferreira JN. Bioprinting salivary gland models and their regenerative applications. BDJ Open. 2024; 10: 39.

[11] Jang JY, Choi N, Ko YH, Chung MK, Son YI, Baek CH, *et al*. Treatment outcomes in metastatic and localized high-grade sali-

vary gland cancer: high chance of cure with surgery and postoperative radiation in T1-2 N0 high-grade salivary gland cancer. BMC Cancer. 2018; 18: 672.

[12] Omitola OG, Iyogun CA. Immunohistochemical study of salivary gland tumors in a tertiary institution in South-South Region of Nigeria. Journal of Oral and Maxillofacial Pathology: JOMFP. 2018; 22: 163–167.

[13] Steuer CE, Hanna GJ, Viswanathan K, Bates JE, Kaka AS, Schmitt NC, *et al*. The evolving landscape of salivary gland tumors. CA: a Cancer Journal for Clinicians. 2023; 73: 597–619.

[14] Jasmer KJ, Gilman KE, Muñoz Forti K, Weisman GA, Limesand KH. Radiation-Induced Salivary Gland Dysfunction: Mechanisms, Therapeutics and Future Directions. Journal of Clinical Medicine. 2020; 9: 4095.

[15] Lou J, Huang P, Ma C, Zheng Y, Chen J, Liang Y, *et al*. Parotid gland radiation dose-xerostomia relationships based on actual delivered dose for nasopharyngeal carcinoma. Journal of Applied Clinical Medical Physics. 2018; 19: 251–260.

[16] Wu VWC, Leung KY. A Review on the Assessment of Radiation Induced Salivary Gland Damage After Radiotherapy. Frontiers in Oncology. 2019; 9: 1090.

[17] Su X, Upadhyay A, Tran SD, Lin Z. Cell-Free Therapies: The Use of Cell Extracts to Mitigate Irradiation-Injured Salivary Glands. Biology. 2023; 12: 305.

[18] Marinkovic M, Tran ON, Wang H, Abdul-Azees P, Dean DD, Chen XD, *et al*. Autologous mesenchymal stem cells offer a new paradigm for salivary gland regeneration. International Journal of Oral Science. 2023; 15: 18.

[19] Upadhyay A, Tran SD. Stem cell therapy for salivary gland regeneration after radiation injury. Expert Opinion on Biological Therapy. 2023; 23: 455–460.

[20] Barazzuol L, Coppes RP, van Luijk P. Prevention and treatment of radiotherapy-induced side effects. Molecular Oncology. 2020; 14: 1538–1554.

[21] Jensen SB, Vissink A, Limesand KH, Reyland ME. Salivary Gland Hypofunction and Xerostomia in Head and Neck Radiation Patients. Journal of the National Cancer Institute. Monographs. 2019; 2019: lgz016.

[22] Wang F, Cai X, Shen Y, Meng L. Cell-scaffold interactions in tissue engineering for oral and craniofacial reconstruction. Bioactive Materials. 2022; 23: 16–44.

[23] Khan E, Farooq I, Khabeer A, Ali S, Zafar MS, Khurshid Z. Salivary gland tissue engineering to attain clinical benefits: a special report. Regenerative Medicine. 2020; 15: 1455–1461.

[24] Sui Y, Zhang S, Li Y, Zhang X, Hu W, Feng Y, *et al*. Generation of functional salivary gland tissue from human submandibular gland stem/progenitor cells. Stem Cell Research & Therapy. 2020; 11: 127.

[25] Mehrabani D, Arshi S, Sadeghi L, Khodabandeh Z, Zare S, Rabiee M. The Ameliorating Effect of Adipose Tissue Stem Cells on Liver Function in Experimental Rats with Liver Fibrosis. International Journal of Nutrition Sciences. 2022; 7: 225–232.

[26] Zanten JV, Jorritsma-Smit A, Westra H, Baanstra M, Bruin-Jellema AD, Allersma D, *et al*. Optimization of the Production Process of Clinical-Grade Human Salivary Gland Organoid-Derived Cell Therapy for the Treatment of Radiation-Induced Xerostomia in Head and Neck Cancer. Pharmaceutics. 2024; 16: 435.

[27] Hosseini-Asl SK, Mehrabani D, Karimi-Busheri F. Therapeutic Effect of Mesenchymal Stem Cells in Ulcerative Colitis: A Review on Achievements and Challenges. Journal of Clinical Medicine. 2020; 9: 3922.

[28] Zhao C, Meng C, Cui N, Sha J, Sun L, Zhu D. Organoid Models for Salivary Gland Biology and Regenerative Medicine. Stem Cells International. 2021; 2021: 9922597.

[29] Jansson PM, Lynggaard CD, Carlander AF, Jensen SB, Follin B, Hoeeg C, *et al*. Mesenchymal stromal/stem cell therapy for radiation-induced salivary gland hypofunction in animal models: a protocol for a systematic review and meta-analysis. Systematic Reviews. 2022; 11: 72.

[30] Grønhøj C, Jensen DH, Vester-Glowinski P, Jensen SB, Bardow A, Oliveri RS, *et al*. Safety and Efficacy of Mesenchymal Stem Cells for Radiation-Induced Xerostomia: A Randomized, Placebo-Controlled Phase 1/2 Trial (MESRIX). International Journal of Radiation Oncology, Biology, Physics. 2018; 101: 581–592.

[31] Paz C, Glassey A, Frick A, Sattar S, Zaorsky NG, Blitzer GC, *et al*. Cancer therapy-related salivary dysfunction. The Journal of Clinical Investigation. 2024; 134: e182661.

[32] Rocchi C, Barazzuol L, Coppes RP. The evolving definition of salivary gland stem cells. NPJ Regenerative Medicine. 2021; 6: 4.

[33] Kimura N, Watanabe T, Fukase M, Wakita A, Noshiro T, Kimura I. Neurofibromin and NF1 gene analysis in composite pheochromocytoma and tumors associated with von Recklinghausen's disease. Modern Pathology. 2002; 15: 183–188.

[34] Luna EB, Montovani PP, Rozza-de-Menezes RE, Cunha KS. Neurofibromin expression by normal salivary glands. Head & Face Medicine. 2021; 17: 5.

[35] Qin L, Wang S. Protective Roles of Inorganic Nitrate in Health and Diseases. Current Medicine. 2022; 1: 4.

[36] Li J, Sudiwala S, Berthoin L, Mohabbat S, Gaylord EA, Sinada H, *et al*. Long-term functional regeneration of radiation-damaged salivary glands through delivery of a neurogenic hydrogel. Science Advances. 2022; 8: eadc8753.

[37] Chou YS, Young TH, Lou PJ. Effects of biomaterial-derived fibroblast conditioned medium on the α-amylase expression of parotid gland acinar cells. Acta Biomaterialia. 2015; 27: 214–223.

[38] Vining KH, Lombaert IMA, Patel VN, Kibbey SE, Pradhan-Bhatt S, Witt RL, *et al*. Neurturin-containing laminin matrices support innervated branching epithelium from adult epithelial salispheres. Biomaterials. 2019; 216: 119245.

[39] Nakao A, Inaba T, Murakami-Sekimata A, Nogawa H. Morphogenesis and Mucus Production of Epithelial Tissues of Three Major Salivary Glands of Embryonic Mouse in 3D Culture. Zoological Science. 2017; 34: 475–483.

[40] Tanaka J, Ogawa M, Hojo H, Kawashima Y, Mabuchi Y, Hata K, *et al*. Generation of orthotopically functional salivary gland from embryonic stem cells. Nature Communications. 2018; 9: 4216.

[41] Tanaka N, Osman AA, Takahashi Y, Lindemann A, Patel AA, Zhao M, *et al*. Head and neck cancer organoids established by modification of the CTOS method can be used to predict in vivo drug sensitivity. Oral Oncology. 2018; 87: 49–57.

[42] Kasamatsu A, Fukushima R, Nakamura K, Kawasaki K, Yoshimura S, Koyama T, *et al*. A novel cell-based transplantation method using a Rho kinase inhibitor and a specific catheter device for the treatment of salivary gland damage after head and neck radiotherapy. Biochemistry and Biophysics Reports. 2022; 32: 101385.

[43] Tatullo M, Marrelli B, Zullo MJ, Codispoti B, Paduano F, Benincasa C, *et al*. Exosomes from Human Periapical Cyst-MSCs: Theranostic Application in Parkinson's Disease. International Journal of Medical Sciences. 2020; 17: 657–663.

[44] Chou SD, Murshid A, Eguchi T, Gong J, Calderwood SK. HSF1 regulation of β-catenin in mammary cancer cells through control of HuR/elavL1 expression. Oncogene. 2015; 34: 2178–2188.

[45] Tanaka J, Mishima K. In vitro three-dimensional culture systems of salivary glands. Pathology International. 2020; 70: 493–501.

[46] Taha EA, Sogawa C, Okusha Y, Kawai H, Oo MW, Elseoudi A, *et al*. Knockout of MMP3 Weakens Solid Tumor Organoids and

Cancer Extracellular Vesicles. Cancers. 2020; 12: 1260.
[47] Ishiguro T, Ohata H, Sato A, Yamawaki K, Enomoto T, Okamoto K. Tumor-derived spheroids: Relevance to cancer stem cells and clinical applications. Cancer Science. 2017; 108: 283–289.
[48] Yoo C, Vines JB, Alexander G, Murdock K, Hwang P, Jun HW. Adult stem cells and tissue engineering strategies for salivary gland regeneration: a review. Biomaterials Research. 2014; 18: 9.
[49] Tanaka J, Mishima K. Application of regenerative medicine to salivary gland hypofunction. The Japanese Dental Science Review. 2021; 57: 54–59.
[50] I T, Kanai R, Hasegawa K, Ogaeri T, Tran SD, Sumita Y. Recent progress in regenerative therapy for damaged salivary glands: From bench to bedside. Oral Diseases. 2024; 30: 38–49.
[51] Fujii M, Sato T. Somatic cell-derived organoids as prototypes of human epithelial tissues and diseases. Nature Materials. 2021; 20: 156–169.
[52] Kretzschmar K, Clevers H. Organoids: Modeling Development and the Stem Cell Niche in a Dish. Developmental Cell. 2016; 38: 590–600.
[53] Lancaster MA, Huch M. Disease modelling in human organoids. Disease Models & Mechanisms. 2019; 12: dmm039347.
[54] Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, *et al*. Human Intestinal Organoids Maintain Self-Renewal Capacity and Cellular Diversity in Niche-Inspired Culture Condition. Cell Stem Cell. 2018; 23: 787–793.e6.
[55] Tatullo M, Marrelli B, Benincasa C, Aiello E, Makeeva I, Zavan B, *et al*. Organoids in Translational Oncology. Journal of Clinical Medicine. 2020; 9: 2774.
[56] Li M, Izpisua Belmonte JC. Organoids - Preclinical Models of Human Disease. The New England Journal of Medicine. 2019; 380: 569–579.
[57] Liu H, Wang Y, Cui K, Guo Y, Zhang X, Qin J. Advances in Hydrogels in Organoids and Organs-on-a-Chip. Advanced Materials (Deerfield Beach, Fla.). 2019; 31: e1902042.
[58] Takebe T, Wells JM, Helmrath MA, Zorn AM. Organoid Center Strategies for Accelerating Clinical Translation. Cell Stem Cell. 2018; 22: 806–809.
[59] Jacob F, Salinas RD, Zhang DY, Nguyen PTT, Schnoll JG, Wong SZH, *et al*. A Patient-Derived Glioblastoma Organoid Model and Biobank Recapitulates Inter- and Intra-tumoral Heterogeneity. Cell. 2020; 180: 188–204.e22.
[60] Zhou Z, Cong L, Cong X. Patient-Derived Organoids in Precision Medicine: Drug Screening, Organoid-on-a-Chip and Living Organoid Biobank. Frontiers in Oncology. 2021; 11: 762184.
[61] Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macro-scale tissue self-organization through organoid bioprinting. Nature Materials. 2021; 20: 22–29.
[62] Dotti I, Salas A. Potential Use of Human Stem Cell-Derived Intestinal Organoids to Study Inflammatory Bowel Diseases. Inflammatory Bowel Diseases. 2018; 24: 2501–2509.
[63] Low JH, Li P, Chew EGY, Zhou B, Suzuki K, Zhang T, *et al*. Generation of Human PSC-Derived Kidney Organoids with Patterned Nephron Segments and a De Novo Vascular Network. Cell Stem Cell. 2019; 25: 373–387.e9.
[64] Muallah D, Matschke J, Kappler M, Kroschwald LM, Lauer G, Eckert AW. Dental Pulp Stem Cells for Salivary Gland Regeneration-Where Are We Today? International Journal of Molecular Sciences. 2023; 24: 8664.
[65] Takata N, Eiraku M. Stem cells and genome editing: approaches to tissue regeneration and regenerative medicine. Journal of Human Genetics. 2018; 63: 165–178.
[66] Phan TV, Oo Y, Ahmed K, Rodboon T, Rosa V, Yodmuang S, *et al*. Salivary gland regeneration: from salivary gland stem cells to three-dimensional bioprinting. SLAS Technology. 2023; 28: 199–209.
[67] Carlander ALF, Gundestrup AK, Jansson PM, Follin B, Hoeeg C, Kousholt BS, *et al*. Mesenchymal Stromal/Stem Cell Therapy Improves Salivary Flow Rate in Radiation-Induced Salivary Gland Hypofunction in Preclinical in vivo Models: A Systematic Review and Meta-Analysis. Stem Cell Reviews and Reports. 2024; 20: 1078–1092.
[68] Gao X, Wu Y, Liao L, Tian W. Oral Organoids: Progress and Challenges. Journal of Dental Research. 2021; 100: 454–463.
[69] Zhang W, Vazquez B, Oreadi D, Yelick PC. Decellularized Tooth Bud Scaffolds for Tooth Regeneration. Journal of Dental Research. 2017; 96: 516–523.
[70] Gao Z, Wu T, Xu J, Liu G, Xie Y, Zhang C, *et al*. Generation of Bioartificial Salivary Gland Using Whole-Organ Decellularized Bioscaffold. Cells, Tissues, Organs. 2014; 200: 171–180.
[71] Maria OM, Zeitouni A, Gologan O, Tran SD. Matrigel improves functional properties of primary human salivary gland cells. Tissue Engineering. Part A. 2011; 17: 1229–1238.
[72] da Silva D, Kaduri M, Poley M, Adir O, Krinsky N, Shainsky-Roitman J, *et al*. Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems. Chemical Engineering Journal. 2018; 340: 9–14.
[73] Kozlowski MT, Crook CJ, Ku HT. Towards organoid culture without Matrigel. Communications Biology. 2021; 4: 1387.
[74] Sumita Y, Liu Y, Khalili S, Maria OM, Xia D, Key S, *et al*. Bone marrow-derived cells rescue salivary gland function in mice with head and neck irradiation. The International Journal of Biochemistry & Cell Biology. 2011; 43: 80–87.
[75] Lim JY, Ra JC, Shin IS, Jang YH, An HY, Choi JS, *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.
[76] Pringle S, Maimets M, van der Zwaag M, Stokman MA, van Gosliga D, Zwart E, *et al*. Human Salivary Gland Stem Cells Functionally Restore Radiation Damaged Salivary Glands. Stem Cells. 2016; 34: 640–652.
[77] Elsaadany B, Zakaria M, Mousa MR. Transplantation of Bone Marrow-Derived Mesenchymal Stem Cells Preserve the Salivary Glands Structure after Head and Neck Radiation in Rats. Open Access Macedonian Journal of Medical Sciences. 2019; 7: 1588–1592.
[78] Al-Serwi RH, El-Kersh AOFO, El-Akabawy G. Human dental pulp stem cells attenuate streptozotocin-induced parotid gland injury in rats. Stem Cell Research & Therapy. 2021; 12: 577.
[79] Nicoletti A, Fiorini M, Paolillo J, Dolcini L, Sandri M, Pressato D. Effects of different crosslinking conditions on the chemical-physical properties of a novel bio-inspired composite scaffold stabilised with 1,4-butanediol diglycidyl ether (BDDGE). Journal of Materials Science. Materials in Medicine. 2013; 24: 17–35.
[80] Kuang T, Zhang M, Chen F, Fei Y, Yang J, Zhong M, *et al*. Creating Poly(Lactic Acid)/Carbon Nanotubes/Carbon Black Nanocomposites with High Electrical Conductivity and Good Mechanical Properties by Constructing a Segregated Double Network with a Low Content of Hybrid Nanofiller. Advanced Composites and Hybrid Materials. 2023; 6: 48.
[81] Tatullo M, Spagnuolo G, Codispoti B, Zamparini F, Zhang A, Esposti MD, *et al*. PLA-Based Mineral-Doped Scaffolds Seeded with Human Periapical Cyst-Derived MSCs: A Promising Tool for Regenerative Healing in Dentistry. Materials. 2019; 12: 597.
[82] Zhou K, Yu P, Shi X, Ling T, Zeng W, Chen A, *et al*. Hierarchically Porous Hydroxyapatite Hybrid Scaffold Incorporated with Reduced Graphene Oxide for Rapid Bone Ingrowth and Repair. ACS Nano. 2019; 13: 9595–9606.
[83] Figliuzzi MM, Giudice A, Pileggi S, Scordamaglia F, Marrelli M, Tatullo M, *et al*. Biomimetic hydroxyapatite used in the treat-

ment of periodontal intrabony pockets: clinical and radiological analysis. Annali Di Stomatologia. 2016; 7: 16–23.

[84] Reddy R, Reddy N. Biomimetic approaches for tissue engineering. Journal of Biomaterials Science. Polymer Edition. 2018; 29: 1667–1685.

[85] Tatullo M, Piattelli A, Ruggiero R, Marano RM, Iaculli F, Rengo C, *et al*. Functionalized magnesium alloys obtained by superplastic forming process retain osteoinductive and antibacterial properties: An in-vitro study. Dental Materials. 2024; 40: 557–562.

[86] Adine C, Ng KK, Rungarunlert S, Souza GR, Ferreira JN. Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands. Biomaterials. 2018; 180: 52–66.

[87] Mansoorifar A, Gordon R, Bergan R, Bertassoni LE. Bone-on-a-chip: microfluidic technologies and microphysiologic models of bone tissue. Advanced Functional Materials. 2021; 31: 2006796.

[88] Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, *et al*. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. Molecular Cancer. 2022; 21: 57.

[89] Revah O, Gore F, Kelley KW, Andersen J, Sakai N, Chen X, *et al*. Maturation and circuit integration of transplanted human cortical organoids. Nature. 2022; 610: 319–326.

[90] Wang Y, Sun Y. Engineered organoids in oral and maxillofacial regeneration. iScience. 2022; 26: 105757.

[91] Tavakol DN, Fleischer S, Vunjak-Novakovic G. Harnessing organs-on-a-chip to model tissue regeneration. Cell Stem Cell. 2021; 28: 993–1015.