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Feature Review

Mouth-Watering Results: Clinical Need, Current Approaches, and Future Directions for Salivary Gland Regeneration

Cecilia Rocchi¹ and Elaine Emmerson ^{1,*}

Permanent damage to the salivary glands and resulting hyposalivation and xerostomia have a substantial impact on patient health, quality of life, and healthcare costs. Currently, patients rely on lifelong treatments that alleviate the symptoms, but no long-term restorative solutions exist. Recent advances in adult stem cell enrichment and transplantation, bioengineering, and gene transfer have proved successful in rescuing salivary gland function in a number of animal models that reflect human diseases and that result in hyposalivation and xerostomia. By overcoming the limitations of stem cell transplants and better understanding the mechanisms of cellular plasticity in the adult salivary gland, such studies provide encouraging evidence that a regenerative strategy for patients will be available in the near future.

Salivary Gland Dysfunction

The salivary glands (SGs) are the organs responsible for keeping the oral cavity lubricated. Saliva is fundamental for efficient mastication, and **hyposalivation** (see [Glossary](#)) leads to **dysphagia**. In addition, a loss of salivary gland function also hinders oral functions, including speaking and taste, due to insufficient lubrication of the oral mucosa [1] (see Clinician's Corner). In addition, saliva has essential antimicrobial properties. As such, the SGs are an essential component of the oral cavity and the host defence against oral pathogens, and hyposalivation ultimately leads to dental caries and fungal and bacterial infections, such as *Candida albicans*. In mammals, saliva is predominantly produced by three pairs of major SGs, located around the jaw: the parotid gland (PG), the submandibular gland (SMG), and the sublingual gland (SLG). The major SGs produce >90% of the total saliva secreted into the oral cavity, with the remaining <10% being produced by the minor labial SGs, found in the lips (reviewed in [2]). In humans, the largest of the major SGs, the PGs, are located inferior and anterior to the ear; the SMGs lie beneath the **mandible**, posterior to the tongue; and the SLGs are found below the oral mucosa of the mouth, anterior to the tongue (reviewed in [3]). The SGs are comprised of various epithelial cells: the secretory acinar cells that produce a serous or mucous liquid [4]; the ductal cells that transport the saliva to the oral cavity [5]; myoepithelial cells that are thought to aid in saliva secretion by constriction [6]; and endothelial cells that make up the gland vasculature [7]; in addition to nerve cells that provide neuronal cues to the gland and play a role in saliva secretion [8], mesenchymal cells that provide growth factors [9], and immune cells [10] (Figures 1A and 2A).

SG dysfunction and hyposalivation often occur as a result of Sjögren's syndrome (SS), radiotherapy, cancer, or ageing. Permanent destruction of the secretory **acini** of the SG or a loss of the supportive signals of the **niche** leads to **xerostomia**, or chronic dry mouth. There is currently no cure and ultimately xerostomia severely impacts patient health and wellbeing [11]. A regenerative strategy would vastly improve the quality of life of many millions of people. In this review, we

Highlights

The vast majority of the 550,000 patients who undergo head and neck cancer radiotherapy annually and more than 4 million patients with Sjögren's syndrome worldwide live with salivary gland dysfunction.

There currently exists no U.S. Food and Drug Administration-approved permanent solution to salivary gland hypofunction and resulting xerostomia.

Stem cell transplant to replace functional salivary gland tissue is an attractive therapeutic option.

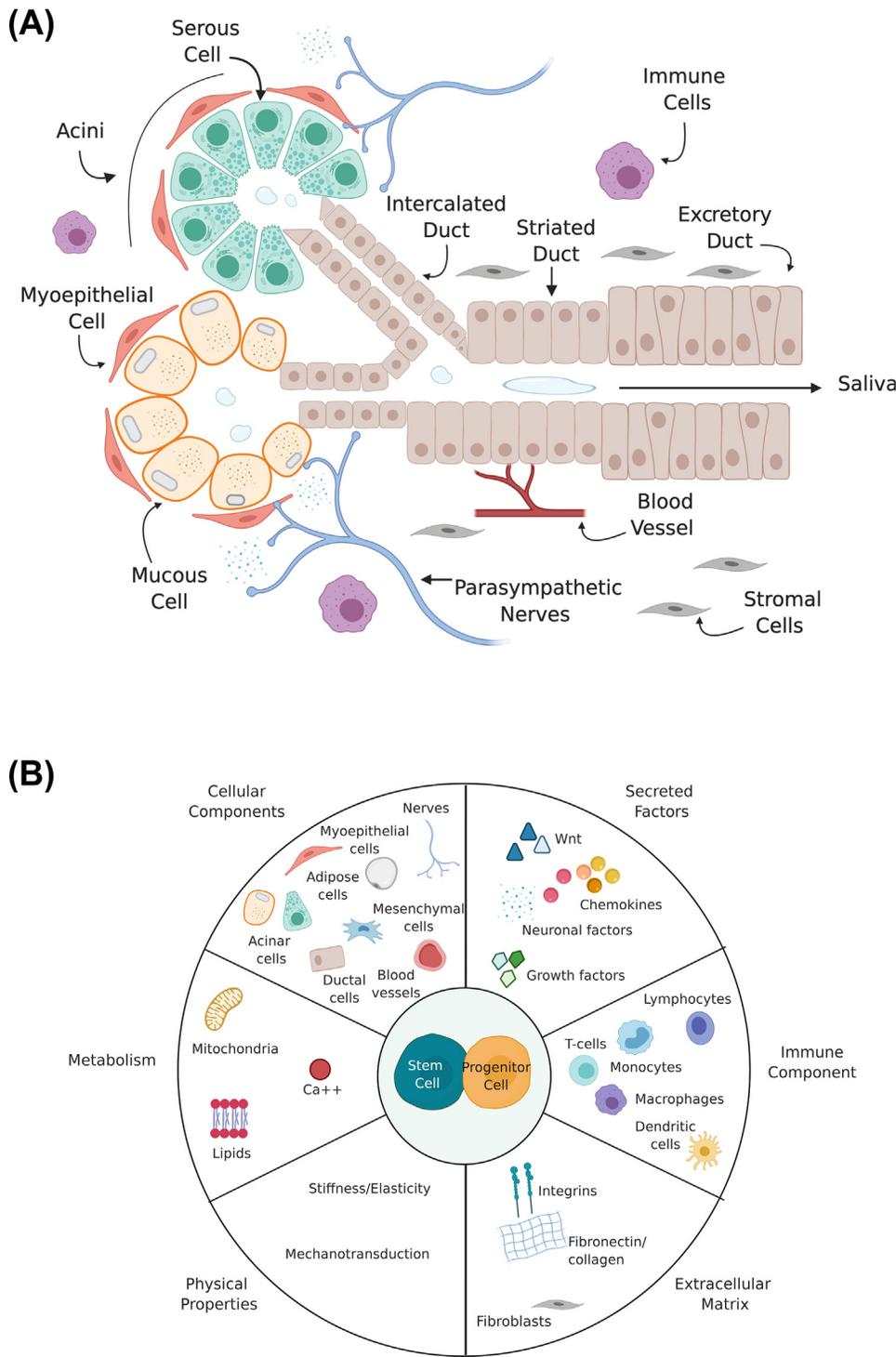
Advances in gene therapy have resulted in a number of studies investigating the replacement of key glandular components as a regenerative strategy.

However, there still exists a clinical bottleneck, with the majority of products or strategies failing to reach phase 4.

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Glossary

- Acini:** the functional saliva-producing unit of the salivary gland.
- Duct ligation:** a technique whereby the major salivary duct is tied off to prevent saliva flow and cause salivary gland injury and degeneration, similar to what would occur with salivary stones.
- Dysgeusia:** altered taste sensation.
- Dysphagia:** difficulty swallowing and chewing.
- Facial palsy:** weakness of the facial muscles, facial drooping or numbness due to reversible/irreversible damage to the facial nerve.
- Fractionated radiation:** radiation split into a number of sessions and given over several weeks. Performed in order to minimise side effects by allowing normal tissues to recover between sessions.
- Hyposalivation:** a substantial reduction in saliva production from all salivary glands or one gland in particular.
- Immune privilege:** cells or tissues that do not elicit an inflammatory immune response from the host.
- iPSCs:** pluripotent stem cells that can be generated from differentiated adult cells via reprogramming.
- Mandible:** lower jaw or jawbone.
- Mimetic:** a drug or compound that mimics or imitates the action of another drug or compound.
- Morphogenesis:** the formation and development of organs and structures within organs.
- Niche:** the microenvironment in which a cell resides. Components of the niche include other cells and tissues, extrinsic cues, growth factors, and matrix components.
- Organoid:** self-organised 3D structures grown from stem cells in an *in vitro* culture and likened to a miniature organ.
- Orthotopically:** occurs in the normal place in the body.
- Paracrine:** cell-to-cell communication whereby a cell produces a signal to induce changes in nearby cells.
- Precision medicine:** specially designed treatments that are most likely to help patients, based on a genetic understanding of their disease.
- Salispheres:** spheroid salivary gland organoids.
- Secretagogues:** substances that promote secretion and salivation.
- Sialadenitis:** infection of the salivary gland.
- Xerostomia:** dry mouth resulting from a change in the composition of saliva or reduced/absent saliva flow.

Figure 1. Cellular Composition of the Salivary Gland and the Stem Cell Niche. (A) The salivary gland epithelium is comprised of serous and mucus acinar cells connected by a network of ductal cells and surrounded by myoepithelial cells,

(Figure legend continued at the bottom of the next page.)

discuss the clinical need for SG regeneration, current treatments, potential approaches that are in development, and future directions.

Clinical Need

Glandular Destruction in Sjögren's Syndrome

Hyposalivation occurs in the chronic systemic autoimmune disease SS following immune-modulated destruction of the SGs over many years [12]. Excessive inflammatory cell infiltration and subsequent elevated cytokine production and tissue proteolysis destroy the saliva-synthesising acinar cells in patients with SS [13] (Figure 2B), leading to SG dysfunction. Destruction of the secretory acini severely impacts the oral health and quality of life of patients, and these patients are at significantly higher risk of developing oral cavities (due to the loss of the antimicrobial effects of saliva). Recent research has implicated stem cell exhaustion as a contributing factor in the loss of epithelial regeneration of the glands [14]. However, advancements in developing treatments for SS have been impeded by the lack of animal models or *in vitro* assays that faithfully recapitulate the human condition.

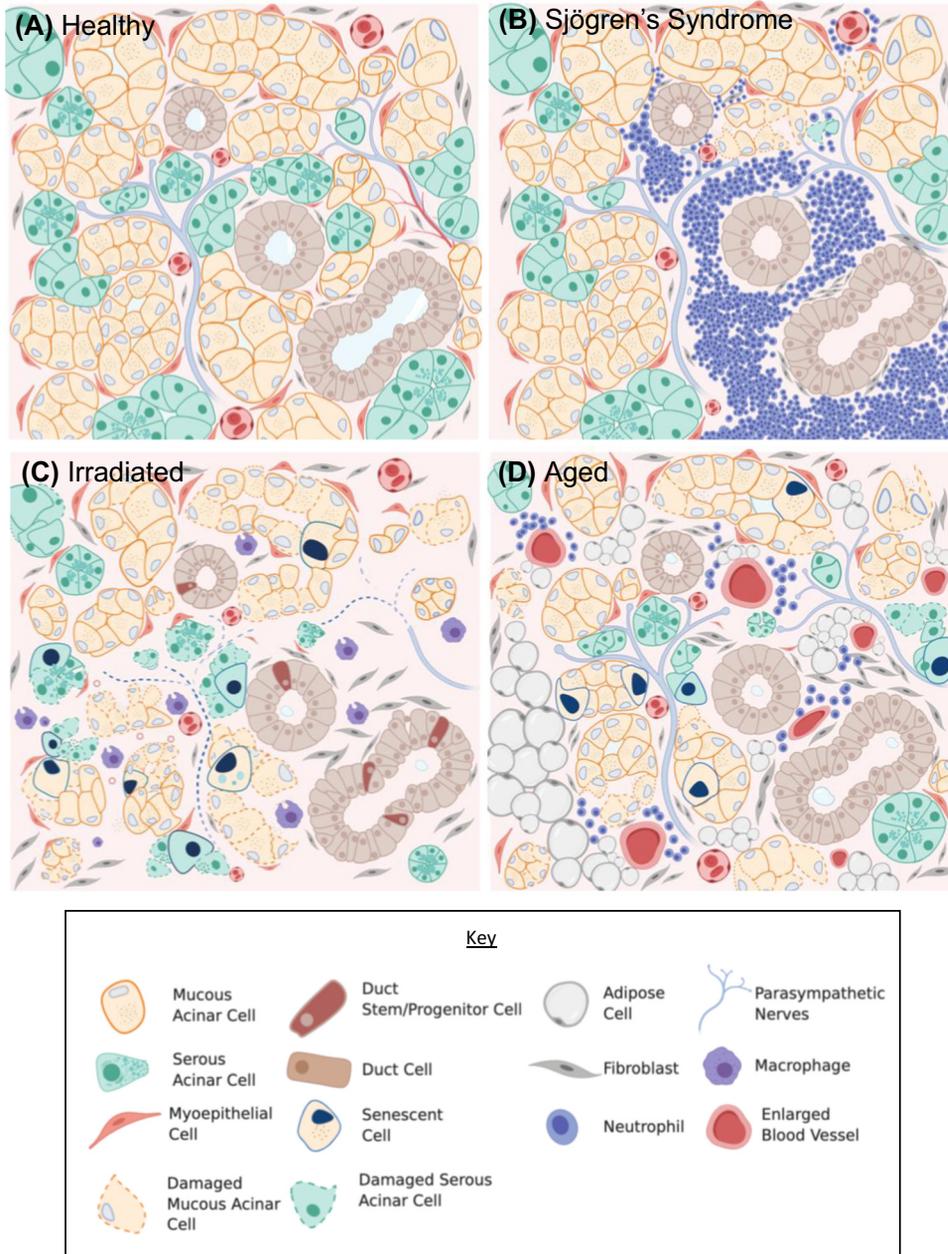
Head and Neck Cancer Radiotherapy

The annual incidence of head and neck cancer is approximately 550,000 new cases, and head and neck cancer causes 300,000 deaths annually. Radiation therapy (RT) remains a lifesaving treatment for cancers of the head and neck. However, the SGs often also lie within the field of irradiation (IR) and thus are inadvertently irradiated along with the tumour [15]. **Fractionated radiation** (2 Gy, 5 d/wk over 5–7 weeks) is a commonly used therapeutic intervention, given as such to reduce the damage to nonmalignant tissue, since the tumour and normal tissue react differently to such a dose [16]. Specifically, normal tissue can repair DNA damage induced by 2-Gy IR better than malignant tissue [17]. Despite the fact that SGs have a relatively slow turnover rate, compared with fast-cycling tissues such as intestine, they respond to radiation injury as would a tissue with a high turnover rate [18,19]. Indeed, soft tissues that are highly vascularised often show acute effects due to IR [20], despite use of a fractionated approach [21]. Cumulative exposure to IR causes extensive destruction of the saliva-producing acini [22] (Figure 2C) and reduced salivary flow rate [17]. Animal experiments have provided a good model of the sequential events that occur following IR; however, methods of analysis have generated conflicting results. Marmary *et al.* [23] report little inflammation, cell apoptosis, or acinar cell loss between 24 hours and 4 weeks post-IR via histological methods. However, such a response is contradictory to the acute SG dysfunction observed and to other studies. Such reports demonstrate (i) elevation of apoptotic markers [24], (ii) loss of acinar cells and a decline in salivary flow rate [24–26], (iii) decrease in amylase secretion [24,25], (iv) plateau, and (v) gland degeneration [25–28]. In addition to a loss of salivary flow, the composition of saliva is also altered following IR, leading to changes in the pH and bactericidal properties of the oral cavity and an altered oral microbiome [29].

Salivary Gland Cancers

Although rare, tumours do arise in the SGs, with an overall incidence of 0.4–3.5 per 100,000 per year in the Western world, where the vast majority are benign (~90%) [30]. In general, surgery to remove the tumour and surrounding tissue (e.g., partial or total parotidectomy) is the preferred management and treatment route [31], as this results in low morbidity and extremely low recurrence rates [32]. However, this can result in a reduction in SG function and dry mouth. In addition, a common side effect (~25%) of parotidectomy is **facial palsy**, due to transient or permanent

stromal cells, and immune cells. The gland epithelium is highly innervated by parasympathetic nerves and vascularised by blood vessels. (B) Stem/progenitor cells within the salivary gland epithelia are supported by the stem cell niche, which includes cellular components such as the nerves, blood vessels, and surrounding mesenchymal cells; secreted factors; inflammatory cells; extracellular matrix; components of metabolism; and physical cues. (Figure drawn using BioRender.)



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Figure 2. Changes in Salivary Gland Composition during Injury and Ageing. Functionality of the salivary gland is preserved by maintaining homeostasis of the gland parenchyma. (A) Homeostasis of the healthy salivary gland epithelium relies on the ability to maintain a fine balance between self-renewal and the differentiation of stem and progenitor cells, as well as maintenance of the healthy status of the components of the niche: nerves, blood vessels, and the mesenchymal microenvironment. (B) In Sjögren's syndrome, the salivary gland parenchyma is subjected to perivascular and periductal infiltration of lymphocytes, with consequent disruption of the glandular niche. This leads to a drastic decline of the acini, followed by reduced saliva production and the development of xerostomia. (C) Therapeutic radiation of head and neck cancer leads to disruption of the salivary gland niche, which includes damage to parasympathetic innervation, leading to dysfunction of the acinar progenitor cells, culminating in the loss of acini. In parallel, infiltration of inflammatory cells, tissues fibrosis, and loss of functionality of the vascular system lead to irreversible disruption of the glandular niche. (D) During ageing, the salivary gland undergoes a slow and steady reduction of acinar cells that are replaced by an increase in the proportion of adipose tissue and tissue fibrosis, leading to a slow decline in the ability to produce saliva. (Figure drawn using BioRender.)

postoperative facial nerve damage [33]. Given the recently documented role of nerve signalling in SG homeostasis and regeneration [24,34], postoperative nerve damage may have a more significant effect on SG function following tumour resection than previously thought.

Ageing

As with many organs, the SGs become less functional with age. Significant age-related decreases are evident in both unstimulated and stimulated saliva secretion in humans [35], and murine models have shown that the stimulated salivary flow rate decreases from 30 weeks of age [36], a result phenocopied in a senescence-accelerated murine model of ageing [37] and mediated by the p16/Ink4a pathway [38]. With increasing age, acinar cells decline and are replaced by adipose tissue and extracellular matrix (ECM) [39]. In addition, the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive apoptotic cells increases in the SMGs with increasing age, suggesting that a contributing factor in a decline in SG function with age is due to changes in cellular replacement and cell death [40] (Figure 2D).

Current Approaches

To track current activity in developing a treatment for xerostomia, we have analysed clinical trials in the National Institutes of Health (NIH) and European databases (https://clinicaltrials.gov/ct2/results?cond=xerostomia&Search=Apply&age_v=&gndr=&type=Intr&rslt=) (Figure 3). While in the last 20 years a considerable investment has been made in preclinical research and clinical trials for xerostomia and dry mouth syndrome, there has only been a modicum of success achieved. In the NIH clinical trials database, there are 134 registered interventional clinical trials (excluding those of unknown status and those whose outcomes do not primarily look at the recovery of SG function) aimed towards the restoration of the damaged SG, with the highest activity reported in the USA and Europe (Figure 3). Among these studies, most do not provide any available results, and only 7% reached phase 4, indicating that the majority of the products/strategies are not moving out of the research pipeline. Of the products that did, those that have been granted U.S. Food and Drug Administration (FDA) approval mainly comprise stimulant medications or **secretagogues** and salivary substitutes or artificial saliva (Figure 3), as described below, that provide temporary release from the discomfort caused by the symptoms. Currently, however, no permanent solution to the problem, the irreversible damage to the functional acinar cells of the gland, exists.

Saliva Substitutes

Saliva substitutes (artificial saliva) are often prescribed to provide temporary relief from xerostomia and in general contain thickening agents and have protective properties. However, in general, saliva substitutes have poor antimicrobial and antifungal properties [41], and no saliva substitute alleviates the symptoms of xerostomia to an acceptable level [42]. The majority of saliva substitutes are composed of rheological modifiers (such as xanthan and guar gums; carboxymethyl cellulose or hydroxyethyl cellulose; glycerol; and mucins, electrolytes, preservatives, and sweeteners), which aim to mimic the rheological properties of saliva. However, the oral microbiome consists of an incredibly complex and diverse composition of bacteria, viruses, fungi, and phages [43], and dysregulation of the oral microbiota may contribute to exacerbating the severity of the side effects associated with IR, such as mucositis [43,44]. No saliva substitutes have yet been able to imitate the antimicrobial properties of saliva, and while they are generally more effective than other treatments for xerostomia, they still only score 2 points on a 10-point visual analogue scale for dry mouth, which is considered as poor [45]. Specifically, commercially available saliva substitutes do not prevent bacterial adhesion [46], an important role that saliva plays under normal conditions to prevent dental decay. Mucins are largely responsible for the lubricating and tissue-protective effects of saliva. Formulations that more closely mirror the antimicrobial properties of saliva, and prevent bacterial adhesion in particular, by replacing mucins, would greatly



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Figure 3. Preclinical and Clinical Approaches for the Treatment of Xerostomia. At the time of writing this review, 301 studies, including both observational and interventional studies, were registered in the National Institutes of Health clinical trial database under the search term ‘xerostomia, dry mouth.’ Only interventional studies with known status and the primary outcome aim of recovery of salivary gland function were taken into consideration for further analysis. The graph shows the number of interventional studies involved in xerostomia development treatment per year, carried out since 1997. Pie charts present their worldwide distribution, the current status of the clinical trial, their distribution in the clinical phases, the type of intervention used, and whether any results have been published.

improve their overall properties. Alternatively, delivery of antimicrobial agents via nanoparticles, as has been demonstrated *in vitro* [47], provides a viable approach to better functionalise artificial saliva.

Oral Rinses, Mouthwashes, and Toothpastes

Oral rinses, mouthwashes, and toothpastes can provide short-term relief from oral dryness and maintain the health of the mouth, teeth, and gums. Such oral rinses are able to significantly increase the volume of saliva production and improve pH buffering [48]. However, like the saliva substitutes described above, these therapeutics merely treat the symptoms for a short period (up to 4 hours) and do not address the underlying clinical problem.

Stimulant Medications

There are various medications available on the market that stimulate any residual salivary tissue to produce saliva and can be used daily. In general, such medications are considered systemic sialagogues and mimic the neuronal signals that stimulate saliva production and secretion from the epithelia. Saliva secretion is controlled by signals from the autonomic nerves [8]. Following head and neck cancer RT, patient-derived human SG biopsies exhibit a loss of parasympathetic innervation [24], a trait phenocopied in murine irradiation models [49], which is hypothesised to be a contributing factor in the loss of salivary secretion. Systemic sialagogues approved by the FDA and the National Institute for Health and Care Excellence include the parasympathomimetic and muscarinic agonists pilocarpine [50] and cevimeline [51], which stimulate residual salivary tissue to secrete saliva. Pilocarpine predominantly signals through muscarinic receptor 1 (M1), while cevimeline signals predominantly through muscarinic receptor 3 (M3). However, because of the widespread expression of these muscarinic receptors throughout tissues and organs of the body, the use of pilocarpine and cevimeline is associated with severe side effects, including nausea [52], diarrhoea, increased urinary frequency, excessive sweating [53], cutaneous vasodilation, bronchoconstriction, hypotension, and bradycardia [54], and patient adherence to the medication is low. Salivary stimulant pastilles, which contain calcium lactate, sodium phosphate, and malic acid (0.76%), are reported to act locally as natural stimulants. However, there are limited published studies on their effectiveness, and while one study demonstrated a significant decrease in xerostomia severity with use, the frequency of xerostomia symptoms remained unchanged [55].

Potential Approaches in Preclinical/Clinical Testing

Stem Cell Transplants

A potential regenerative approach, not only for SGs but also for organs as a whole, is through the use of stem cell therapy [56]. Stem cell therapy-based approaches account for 11.9% of the clinical interventions for xerostomia (Figure 3). While the progress of stem cell clinical trials for xerostomia is encouraging, given that the majority are in early phase 1 or 2, these approaches rely solely on the use of mesenchymal stem/stromal cells (MSCs) (reviewed in [57]).

Mesenchymal Stem/Stromal Cells

MSCs are an attractive source of stem cells due to their relative abundance and the ability to harvest them in a noninvasive manner. In the majority of *in vivo* MSC salivary transplant studies that have been published, MSCs have been harvested from bone marrow, adipose tissue, or umbilical cord blood [58–69], or the studies have involved isolating peripheral blood mononuclear cells (PBMCs) and differentiating them into effectively conditioned PBMCs (E-MNCs) [70] (Table 1). However, MSCs have limitations that preclude their widespread adoption as a stem cell therapy, with the primary concern being safety. There is yet to be any report of the long-term safety of MSCs, and because of their **immune privilege**, they have the potential to metastasise to other tissues or organs. In addition, their use has been associated with tissue fibrosis, and their heterogeneous nature and donor-dependent efficacy pose a significant hurdle [71]. As such, the identification of adult tissue-resident stem cells that can regenerate functional SG tissue will be of considerable benefit to the field.

Table 1. Stem Cell Transplants as a Salivary Gland Regenerative Approach

Donor	Stem cell type	Recipient	Disease (model)	Details	Key results	Refs
C57BL/6 mice	BMdMSCs	C57BL/6 mice	RIX	1×10 ⁵ MSCs injected into each SMG 24 h post-IR (15 Gy)	Cell integration after 4 wk. Significant increase in salivary flow, increased functional acinar cells, reduced apoptosis, and increased vasculature.	[58]
C57BL/6-Tg (CAG-EGFP) mice	AdMSCs	C57BL/6 mice	RIX	1×10 ⁵ MSCs injected into each SMG 10 wk post-IR (10 Gy)	Cell integration into ductal and endothelial cells. Improved salivary flow and increased angiogenesis.	[59]
C57BL/6 mice	BMdMSCs	NOD. SCID- <i>Prkc</i> ^{SCID} mice	RIX	1×10 ⁵ MSCs injected into each SMG 11 d post-IR (15 Gy)	Significant increase in salivary production; increased body weight and gland weight.	[60]
C57BL/6-TgH/BALB/c mice	BMdMSCs	NOD mice	SSLMM	1×10 ⁵ MSCs injected IV at 6 or 16 wk of age	Improved salivary flow, reduced lymphocytic infiltration, reduced T and B cells, and increased Tregs.	[61]
Balb/c or C57BL/6-gfp mice	BMdMSCs	NOD/Ltj mice	SSLMM	1×10 ⁵ MSCs injected IV at 6 or 16 wk of age	Cell integration. Reduced inflammation, T-cell polarisation toward Treg and Th2, while suppressing Th17 and Tfh responses, improved saliva production.	[62]
C57BL/6JJcl or C57BL/6-Tg (CAG-EGFP) mice	PBMNCs (differentiated into E-MNCs)	C57BL/6JJcl mice	RIX	5×10 ⁴ E-MNCs injected into each SMG 3 d post-IR (12 Gy)	Cell engraftment into glandular and vascular tissues. Improved saliva production, reduced inflammatory genes, increased stem cell markers, cell proliferation, and blood vessel formation.	[70]
C57BL/6-TgN mice	SGSCs	C57BL/6 mice	RIX	Single cells (60,000–90,000), SG spheres (4000–7000), KIT ⁺ cells (300–1000), or KIT ⁻ cells (10,000–90,000) injected into each SMG 30 d post-IR (15 Gy)	Cell integration and formation of ductal structures at the injection site and mucin-positive cells throughout the gland (after 90 d). Increased cell proliferation, increased acinar cells, and significantly improved saliva production.	[72]
C57BL/6 mice	SGSCs	C57BL/6 mice	RIX	400 KIT ⁺ cells, 1000 KIT ⁺ CD24 ⁺ cells, or 400 KIT ⁺ CD24 ⁺ CD49f ⁺ cells injected into each SMG 30 d post-IR (15 Gy)	Improved saliva production (90 d post-transplant), increased expression of ductal markers, rescued vascularisation, and reduced fibrosis.	[73]
C57BL/6 mice	SGSCs	C57BL/6 mice	RIX	5000 CD133 ⁺ , 150 KIT ⁺ CD49f ⁺ , or 5000 CD24 ⁺ CD29 ⁺ cells injected into each SMG 30 d post-IR (15 Gy)	Increased saliva production (120 d post-transplant), with all cell transplants compared with control (however, no significance reported). Most substantial improvement with CD133 ⁺ or CD24 ⁺ CD29 ⁺ cells.	[74]
C57BL/6-Tg (CAG-EGFP)1OsB/J and B6.Cg-Tg (CAG-DsRed*MST) 1Nagy/J mice	SGSCs	C57BL/6 mice	RIX	5000 cells injected into each SMG 30 d post-IR (15 Gy)	Donor cell (GFP ⁺) engraftment. Improved saliva production (60, 90, and 120 d post-transplant; 46%±2.11%). Reappearance of functional acinar cells (visualised by histology and AQP5 immunostaining). No sign of cell transformation or tumourigenesis.	[75]

Table 1. (continued)

Donor	Stem cell type	Recipient	Disease (model)	Details	Key results	Refs
NOD.Cg-Tg (CAG-DsRed ⁺ MST) 1Nagy/J, B6.129P2-Lgr5 ^{tm1} (cre/ESR1) ^{Cle} /J and LGR6-EGFP mice	SGSCs	C57BL/6 mice	RIX	100, 1000, or 10,000 cells injected into each SMG 28 d post-IR (15 Gy)	Donor cell (RPF ⁺) engraftment. Improved saliva production (120 d post-transplant; range 53%±8% to 79%±6%, depending on number and passage of cells). Reappearance of functional acinar cells (visualised by histology). No sign of cell transformation or tumourigenesis.	[78]
Humans	SGSCs	NSG mice	RIX	500, 5000, or 50,000 salisphere cells injected into each SMG 30 d post-IR (5 Gy)	Survival and proliferation of injected cells. Generation of acinar and ductal cells, which are positive for AQP5, amylase, and keratins. Significant increase in saliva production and increase in gland weight.	[83]
Humans	AdMSCs	C3H mice	RIX	1×10 ⁶ MSCs injected IV (tail vein) immediately after IR (15 Gy) and every wk thereafter for 3 wk	Increased saliva production, increase in mucin-producing acinar cells, increased amylase production, and decreased fibrosis. Reduced TUNEL ⁺ apoptotic cells.	[65]
Humans	AdMSCs	C57BL/6 mice	RiIX	1×10 ⁵ MSCs injected into each SMG on day 28	Presence of human RNA and cell engraftment 16 weeks post-transplant. Increased saliva production and reduced apoptosis.	[68]
Humans	AdMSCs	Sprague-Dawley rats	RIX	1×10 ⁶ MSCs injected SC into each SMG immediately after IR (18 Gy)	Improved salivary flow, improved angiogenesis, elevated expression of VEGF, reduced apoptosis, and reduced fibrosis.	[66]
Humans	UCdMSCs	Humans	SS	One dose of 1×10 ⁶ MSCs per kg body weight injected IV	Improvements in Sjögren's Syndrome Disease Activity Index scores and improved salivary flow rate (at 2 wk and 1 mo post-transplant). Abolished production of anti-SSA/Ro in serum.	[62]
Humans	AdMSCs	Humans	RIX	2.8×10 ⁶ MSCs injected into each SMG at least 2 y after radiotherapy/chemoradiotherapy	Improvements in salivary flow rate (at 1 and 4 mo post-transplant), amelioration of the symptoms of xerostomia. Increased serous cells and reduced adipose and connective tissues.	[67,69]

AdMSCs, Adipose-derived mesenchymal stem cells; AQP5, aquaporin 5; BMdMSCs, bone marrow-derived mesenchymal stem cells; EGFP, enhanced GFP; E-MNCs, effectively conditioned mononuclear cells; IR, irradiation; IV, intravenous; MSC, mesenchymal stem/stromal cell; NOD, nonobese diabetic; PBMNCs, peripheral blood mononuclear cells; RiIX, radioiodine-induced xerostomia; RIX, radiation-induced xerostomia; SCID, severe combined immunodeficiency; SG, salivary gland; SGSCs, salivary gland stem cells; SMG, submandibular gland; SS, Sjögren's syndrome; SSA, Sjögren's syndrome-related antigen A; SSLMM, Sjögren's syndrome-like mouse model; Tfh, T-follicular helper cell; Tg, transgenic; Th, T-helper cell; Treg, regulatory T cell; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labelling; UCdMSCs, umbilical cord-derived mesenchymal stem cells; VEGF, vascular endothelial growth factor.

Enrichment of Salivary Gland-Derived Stem Cells

Identification of cells in the adult SG that exhibit stem cell characteristics and are able to rescue IR-induced hyposalivation when transplanted *in vivo* is encouraging evidence of the existence of adult tissue-resident stem/progenitor cells. While thus far the isolation of murine salivary gland stem cells (SGSCs) has been mainly based on markers of adult stem cells identified in other tissues, transplantation of these cells has provided a proof of concept that adult SGSCs could be a viable approach to treating xerostomia. The *in vivo* functionality of murine SGSCs was first demonstrated with selected primary sphere-derived KIT⁺ cells [72]. In brief, cell suspensions were prepared by mechanical and enzymatic digestion of donor mouse SMGs in collagenase II, hyaluronidase, and CaCl₂ and incubated in supplemented growth medium to generate spheres. Female recipient mice that had previously been irradiated with 15 Gy were transplanted with 60,000–90,000 SGSC spheres after 3 days in culture via intraglandular injection 30 days post-IR. While transplant of these cells significantly improved saliva production in 42% (5 of 12 animals) compared with irradiated nontransplanted mice, later studies showed that selection and enrichment of CD24⁺CD29⁺ SG-derived cells improved regeneration of the acinar compartment and allowed up to 70% rescue of normal SG function compared with nonirradiated control [73].

Furthermore, the development of a SG-derived **organoid** culture allowed not only expansion of rare SGSCs [73–75] but also the investigation and characterisation of key niche signalling components necessary for SG homeostasis and regeneration [76–79]. WNT stimulation of SG organoid cultures results in the expansion of the SG stem/progenitor pool without the use of stem cell markers. These WNT-driven organoids contain heterogeneous populations of stem/progenitor cells, as well as differentiated SG cells, which, when transplanted in irradiated recipient mice, resulted in a significant improvement in saliva flow over those previously reported [78].

Since no common marker of murine and human SGSCs has been demonstrated, and because bottlenecks often arise when translating findings from mouse to human, the possibility of expanding functional cell types without the need for selecting for a specific stem cell marker might be crucial when translating to human studies. Thus, better understanding the complexity of the stem cell niche will be essential for the progression of stem cell therapies to human patients. Table 1 presents the details of pivotal published SGSC transplant experiments.

Limitations of the Stem Cell Transplant Approach

IR is known to induce quiescence and dormancy in SGSCs [80,81], although it may be possible to overcome this by providing the correct stimuli to reactivate cells [80]. However, the relatively advanced average age of patients with head and neck cancer at diagnosis (63–65 years of age), together with the natural decline of adult tissue stem cells from aged SGs [77,78,82], may pose a limiting step in the application of human SGSCs for xerostomia treatment. While the number of SGSCs seems to increase in number in the SGs of old mice, their ability to form primary spheres is drastically reduced [78], indicating that a better understanding of how the niche signals to these cells will be crucial in order to reactivate and expand the resident stem/progenitor cell pool.

Moreover, while murine studies have provided compelling evidence that cells within the SGs have regenerative potential, further experiments are required to establish if human SGSCs can regenerate functional tissue in human patients with xerostomia and provide long-term salivary rescue. The majority of murine studies discussed above have used KIT, CD24, and CD29 as markers of SGSCs. Although these markers appear to be present in the human SG [77, reviewed in 83], their regenerative potential still needs to be investigated, and their true stem cell nature is yet to be conclusively demonstrated. To date, only one published study has analysed SG regeneration following human SGSC transplant [83]. However, this study demonstrated salivary rescue following an

IR dose of 5 Gy, which is considerably lower than the 10–15 Gy doses used in all other published studies. Whether human SGSC transplant has the same success at a higher, more clinically relevant dose is unknown.

Finally, development of an autologous stem cell isolation and transplant protocol will require cells to be stored for a period of time following isolation before the post-IR gland is ready for transplant. Cryopreservation and storage of rat SGSCs for 3 years provided encouraging evidence that autologous transplants could be a viable therapeutic approach [84]. However, in the absence of any published data, the *in vivo* functionality of cryopreserved SGSCs remains to be confirmed.

Tissue-Resident Stem Cells

An alternative approach to stem cell transplant would be to promote endogenous stem/progenitor cells (e.g., SOX2⁺, KRT14⁺, KRT5⁺) or terminally differentiated cells (e.g., MIST1⁺) to replenish tissue. Recent *in vivo* lineage-tracing studies have provided evidence that different lineages within the glands are maintained by distinct stem/progenitor cell populations [24,26,28,85–89]. In the SMG, MIST1⁺ lineage tracing provides evidence that acinar cell self-duplication maintains gland homeostasis [90], while in the SLG, SOX2⁺ cells give rise to acinar cells during homeostasis and following IR injury [24]. Conversely, KIT⁺ cells are long-lived progenitors that give rise to intercalated ductal cells [24,88], KRT14⁺ cells divide asymmetrically to give rise to larger granular ducts [86,88], and a population of overlapping but nonidentical KRT5⁺ and AXIN2⁺ cells produces intercalated and excretory ductal cells [28]. In addition, p63⁺ cells replace basal ductal cells [26], and SMA⁺ myoepithelial cells replenish themselves via self-renewal [26,88].

Transdifferentiation of Tissue-Resident Cells

Cellular plasticity and the ability of once thought to be lineage-restricted cells to become other cell types have recently become evident in epithelial organs that are susceptible to severe injury, such as the liver [91,92] and intestine [93]. While evidence demonstrates that, in the SMG, MIST1⁺ acinar cells replace themselves during homeostasis [90] and following IR [28], after severe injury (15 Gy IR), lineage tracing demonstrates that both KRT5⁺ and AXIN2⁺ cells are also able to replace acinar cells (confirmed by the markers aquaporin 5 [AQP5], MIST1, Na-K-Cl cotransporter, and inositol 1,4,5-triphosphate receptor 3) [28]. Conversely, stress and/or excessive inflammation is known to induce acinar-to-ductal metaplasia in the exocrine pancreas [94], and ductal ligation of the SMG results in reversible acinar cell atrophy and transient transdifferentiation of acinar cells to ductal cells [95], demonstrating a level of cellular plasticity in the SMG upon severe injury, although the mechanism by which this occurs is still unknown. There is evidence of transcription factor modulation resulting in plasticity, by cell-autonomously modulating gene expression in numerous secretory organs that lack regenerative potential under normal homeostatic conditions, such as the pancreas [96]. Accordingly, overexpression of *Sox10* in salivary gland epithelial duct (SIMS) and human adult mammaglobin (MMG) luminal duct (MCF10A) cell lines induces expression of *Kit*, and when cultured in 3D conditions, these cells develop organoid-like structures with K19⁺ (ductal), SMA⁺ (myoepithelial), and CD166⁺ (acinar) cells [97]. Thus, transdifferentiation of mature cells to a different cell fate occurs under severe injury conditions and may provide a means to therapeutically regenerate salivary epithelia to restore gland function. It appears that both cell-autonomous and cell-nonautonomous mechanisms control cellular plasticity, and signals from the stem cell niche, such as WNT signals in the intestine [98], may control dedifferentiation and promote plasticity following injury. Thus, understanding the stem cell niche is an essential part of regenerative medicine.

Manipulation of the Stem Cell Niche to Stimulate Regeneration

An alternative or additional approach to exogenous regeneration of damaged tissue is manipulation of the cellular environment, or niche, to provide a more hospitable environment to promote

endogenous regeneration, cellular plasticity, and transdifferentiation or to support successful cell engraftment. The IR-injured SG, for example, is generally considered, at different stages of the repair process, to be inflamed, senescent and fibrotic, and to exhibit vascular and nerve alterations. There are multiple aspects of the SG stem cell niche that are known to be essential for development or regeneration that could be targeted. As discussed above, the mammalian SG stem/progenitor cell niche comprises multiple signals during homeostasis, including the cellular components, such as the nerves, blood vessels, and surrounding mesenchymal cells; secreted factors; inflammatory cells; ECM; components of metabolism; and physical cues (Figure 1B). Recent advances in regenerative medicine are also focussing on niche manipulation as a means to promote SG regeneration.

While the regulation of saliva secretion by neuronal signals is well documented [8], the role that nerve signals play in SG regeneration had, until recently, been overlooked. The murine SMG requires neuronal signals from the parasympathetic ganglion during **morphogenesis** [99], and the SMG and SLG do not develop properly in mice lacking craniofacial nerves (*Phox2b*-null mice) [100]. In contrast, the acetylcholine **mimetic** carbachol (CCh) is sufficient to rescue branching morphogenesis in the absence of nerves [100]. The adult rabbit SMG undergoes degeneration and a loss of functional markers, including the water channel, AQP5, upon denervation, a functional change that is reversible upon reinnervation [34]. In the adult mouse, surgical denervation of the SMG and SLG, by transection of the chorda tympani, results in a substantial reduction in the number of SOX2⁺ progenitor cells and a significant reduction in the extent of acinar cell replacement by SOX2⁺ cells in the SLG [24]. Furthermore, this study demonstrated that the addition of a muscarinic mimetic was sufficient to increase SOX2⁺ cell proliferation and drive acinar cell regeneration [24]. Future studies that test the effect of novel muscarinic mimetics to drive *in vivo* SG regeneration following irradiation injury, such as those undertaken in a mouse model of multiple sclerosis [101], may prove clinically beneficial.

In addition to neuronal factors, there are a number of growth factors that play a role in SG development and regeneration (reviewed in [102]). Glial cell-derived neurotrophic factor (GDNF) is highly expressed in murine SGSCs that express KIT and Sca-1 [103]. Furthermore, GDNF treatment leads to an increased number of acini and improves saliva production after radiation injury [103]. Crucially, this effect was attributed to increasing stem cell proliferation, since GDNF also enhanced sphere formation in cultured SGSCs. However, the authors did not note any increased proliferation of the head and neck cancer cell line, SCC 22A, or head and neck tumours *in vivo*, demonstrating the therapeutic potential of such an extrinsic signal [103]. However, GDNF does not demonstrate any radioprotective properties on salivary stem cells [103,104], suggesting that the observed effects are modulated via improved cell proliferation [103] or indirectly as a result of improvements to the innervation of the gland [24].

WNT signalling plays a role in embryonic patterning and organ development, cell proliferation and migration and differentiation, cell fate and polarisation, and apoptosis in multiple mammalian organ systems (reviewed in [105]). WNT signalling is essential for branching morphogenesis during SG development [106], and its expression is upregulated in the ducts of the adult murine SMGs during regeneration following **duct ligation** injury [107]. Of importance to regenerative strategies, the transient activation of WNT in a murine model prevents IR-induced SMG damage by preventing apoptosis and preserving expression of the confirmed and putative stem cell markers *Ascl3* and *Lgr5* [108]. Local delivery of WNT [109] may offer a therapeutic strategy to improve SG regeneration in the future. However, given that the activation of the WNT/ β -catenin pathway via a β -catenin gain-of-function murine model induces a rapidly growing, aggressive SG squamous cell carcinoma [110], the optimisation of regeneration without tumourigenesis will require careful balance.

Proper vascularisation is essential for endogenous regeneration and for stem cell graft success. While the majority of murine SMG regeneration studies do not report effects on the vascular system, both the drug deferoxamine and laminin- and growth factor-loaded hydrogels improve vascularisation during SMG regeneration [111].

Finally, the mechanical environment of the IR-damaged SG likely plays a role in regenerative ability. The mouse and human SMGs undergo fibrosis following radiation [112,113]; however, how SGSCs survive and thrive in stiff environments, which recapitulate post-irradiation fibrosis, is yet to be investigated. Epithelial cell behaviour can be heavily influenced by ECM stiffness in multiple tissues during development, regeneration, and tumourigenesis. Moreover, murine SG explants undergo aberrant morphogenesis when cultured in a stiff matrix [114], implying that tissue stiffness will influence stem cell-mediated regeneration. Scaffolds or delivery mechanisms that are designed for optimal stiffness will likely be crucial to successful graft survival.

Bioengineering Approaches

Another regenerative strategy for SG dysfunction is through the use of bioengineering. Isolated SG cells cultured with the 3T3 cell line were able to engraft into the murine SMG, particularly around the ducts, following ductal ligation injury [115]. However, the engraftment efficiency of salivary cells into recipient tissue is far from optimal, and as such, a number of studies have been undertaken to optimise the delivery method and cellular environment to improve engraftment.

Tissue scaffolds have proved to be useful for bioengineering purposes in multiple organs [116,117]. Optimal 3D salivary cultures aim to recapitulate polarisation and the secretory nature of cells (reviewed in [118]); however, following the generation of acini in a 3D culture in Matrigel, the human SG cell line, HSG, undergoes extensive cell death, suggesting Matrigel alone is insufficient for the full maturation and long-term survival of such structures [119]. 3D **salispheres** cultured in fibrin hydrogel and Matrigel, and supplemented with epidermal growth factor and insulin-like growth factor-1 (IGF-1), produced mature-looking structures capable of amylase production [120], while human salivary cells cultured in a 3D system express differentiation markers AQP5, occludin, and amylase and polarity markers ZO-1 and claudin-1 [121]. Moreover, decellularised rat SMGs seeded with rat SMG cells demonstrated successful integration into donor SMGs, with expression of the differentiation markers AQP5, occludin, and amylase [122].

Delivery of Bioactive Compounds

The administration of bioactive compounds directly into the SG is a promising therapeutic option. Retroductal delivery, by injecting into the major duct, has previously been undertaken to administer a multitude of reagents, including growth factors, primary cells, adenoviral vectors, cytokines, and antioxidant compounds [23,123–133]. Donor rat SG cells, also administered through the major duct, are reported to be able to incorporate into the SG epithelia, although the evidence for this provided in the publication is limited to histology showing donor cells that have persisted 21 days after transplant [127]. Administration of basic fibroblast growth factor (bFGF) significantly increased cell proliferation of both acinar and ductal cells following duct ligation injury and during normal homeostasis; however, the dose required to elicit effects in a normal homeostatic gland was over 50 times more than in an injured gland, suggesting that bFGF can promote tissue repair in the murine SMG [124]. Similarly, mice administered IGF-1 exhibited improved stimulated salivary flow and amylase production 30 days post-IR, in addition to activation of Akt and a complete rescue of salivary function to a level comparable with that of nonirradiated control animals by 60 days [125,126]. Nanoparticles also provide a viable preventative approach and can be delivered to the murine SMG via the major duct, the Wharton's duct [133]. Nanoparticles that are able to silence the proapoptotic gene, *Pkcδ*, via the use of siRNAs were efficient at

reducing the number of apoptotic cells following IR-induced salivary damage and improved salivary secretion after 3 months, implicating a radioprotective effect [132]. Systemic histamine administration also protects SMGs from IR-induced apoptosis and maintains saliva secretion [134]. Table 2 outlines the details of published studies where bioactive compounds have been administered to the SG as a therapeutic measure.

Gene Therapy Approaches

A loss of functional water channels in SG epithelia is often considered one of the hallmarks of SG dysfunction, and recent advances in the field have aimed to restore permeability in an attempt to increase salivary production. In 1997, the first SG gene therapy study demonstrated a method to deliver the human *AQP1* gene to the SMG of donor Wistar rats via recombinant adenovirus delivery (AdhAQP1) [128]. In this study, hAQP1 expression was localised to both acinar and ductal cells and, following IR, led to a two- to threefold increase in salivary secretion compared with that of a control virus, suggesting that hAQP1 delivery may be a promising therapeutic approach to restore SG function after IR injury. Follow-on studies demonstrated similar efficacy in miniature pigs [129], while results in rhesus monkeys were less consistent (two of four showed improved salivary flow) [130]. Similarly, targeted increase of glandular permeability by AQP1 AAV2 gene therapy in a murine model of SS improved salivary flow and reduced inflammation, specifically T-cell infiltrate [131], implicating the positive effect that gene therapy may have in the treatment of patients with SS. At the date of publishing this review, both preclinical work investigating AAV-AQP1 as a means to restore SG function in patients with SS and a phase 1 dose escalation clinical study to treat SG dysfunction in patients with grade 2 or 3 IR-induced xerostomia (ClinicalTrials.gov identifier NCT00372320) are being undertaken by the National Institute of Dental and Craniofacial Research (NIDCR), the rights of which have been recently acquired by MeiraGTx. In the first published study from the trials, 11 patients who had previously undergone RT were assessed following AdhAQP1 vector delivery to the PG. All subjects tolerated vector delivery, and there were only minimal mild to moderate adverse effects and no deaths reported. Five subjects experienced a subjective improvement in their symptoms of xerostomia, indicating that the gene therapy is safe and well tolerated and that it improved PG salivary flow in a subset of patients [135]. Moreover, these five patients experienced elevated salivary flow 3–4.7 years after treatment, with improved symptoms for ~2–3 years [136]. Of importance, many AAV serotypes will be viable vectors in any future gene therapy approaches [137].

Neutralisation of inflammatory mediators via gene therapy is also a potential therapeutic approach for patients with SS. Neutralisation of B-cell-activating factor and proliferation-inducing ligand in an animal model of SS led to significantly reduced CD138⁺ inflammatory cells and a reduction in IgG and IgM levels in the SG; however, salivary flow was unaffected [138]. In addition to replacing missing components of the SG environment, gene therapy can be used to administer protective growth factors. Delivery of human keratinocyte growth factor (hKGF) via adenoviral vector was protective against IR-induced SMG dysfunction, and salivary flow, measured following pilocarpine stimulation, was similar in mice administered the AdLTR(2)EF1 α -hKGF vector compared with nonirradiated control animals [123]. Table 2 outlines the details of published studies where gene therapy has been used as a therapeutic measure for SG dysfunction. However, while encouraging results have been observed, gene therapy also has its challenges, including the risk of the recipient mounting an immune response, and the occurrence of inflammation, off-target effects, and insertional mutagenesis.

Future Approaches

While significant progress has been made in moving toward a regenerative strategy for xerostomia, some hurdles remain. One aspect that has received little attention is how the cellular

Table 2. Delivery of Bioactive Compounds or Gene Therapy to the Salivary Glands as a Regenerative Approach

Bioactive compound/gene	Delivery mechanism	Recipient(s)	Disease (model)	Details	Key findings	Refs
Rat acinar cell 'slurry'	Primary cell delivery	Sprague-Dawley rats	None	250- to 300- μ L cell slurry delivered via SMG ductal cannulation	Donor cells persisting after 21 d post-transplant. However, claims of cytodifferentiation into acinar and ductal cells not backed up by specific cell labelling.	[127]
hKGF	Adenovirus	CH3 mice	RIX	10^8 – 10^{10} particles per gland pre-IR (single dose of 15 Gy or fractionated dose of 6 Gy for 5 d) delivered intraductally to SMG	Secreted hKGF detectable in murine SMGs after 9 wk. Improved salivary flow rate and maintained body weight after 9 wk. Increased cycling (BrdU ⁺) cells, no difference in AQP5 ⁺ cells, nonsignificant trend toward increased KIT ⁺ cells. No effect on (SCC) tumour growth.	[123]
hrbFGF	Growth factor delivery	Wistar rats	Ligation-induced SG damage	0.01, 0.1, 1.0, and 10 ng/gland 2 wk postligation delivered via ductal cannulation to SMG	SMG weight increase, recovery of acini, increase in PCNA ⁺ cells, and reduction in fibrosis.	[124]
mNKCC1 or mPkc δ	siRNA delivery	BALB/c/cByJ mice	RIX	4 μ g siRNA/gland pre-IR (10 Gy) delivered via retroductal injection to SMG	siRNA knockdown of Nckk1 results in decreased saliva secretion (~60%), but no increase in apoptosis, no change in histology or acinar cells, and no change in body weight. siRNA knockdown of Pkc δ blocked the upregulation of Pkc δ mRNA usually seen following IR, coupled with an improvement in morphology and increased AQP5 ⁺ acinar cells at 90 d and reduced apoptosis (by 70%) at 48 h after IR. Rescue of saliva secretion (80% \pm 26% of control).	[132]
mIGF-1	Growth factor delivery	FVB mice	RIX	5 μ g recombinant IGF-1, days 4–8 post-IR (5 Gy or 2 Gy per day for 5 d) delivered IV (tail vein). Analysis of PG.	Restoration of salivary flow rates (72%, 93%, and 81% of control on days 30, 60, and 90, respectively). Increase in AMY1 ⁺ cell area and amylase protein in saliva and reduction in apoptosis.	[125,126]
IL-6, HIL-6, or anti-IL-6	Monoclonal antibody or neutralising monoclonal antibody delivery	C57BL/6, IL-6-ko, or sgp130Fc mice	RIX	100 μ g IL-6, HIL-6, or anti-IL-6 administered 2 wk pre-IR (13 Gy (single fraction) or 28 Gy in five daily fractions of 5.6 Gy), delivered IV.	No difference in induction of γ H2AX (DNA damage) up to 48 h but substantially decreased 48–72 h with IL-6 pretreatment compared with control. Up to threefold increase in saliva production and a reduction of p21 ⁺ acinar cells with IL-6 or HIL-6 pretreatment at 8 wk post-IR. Rescued salivary production 8 wk post-IR with anti-IL-6.	[23]
Histamine	Compound delivery	Sprague-Dawley rats	Whole body IR	Daily histamine injections (delivered SC, 0.1 mg/kg) starting 24 h before IR (5 Gy, whole body).	Rescue of saliva production compared with IR alone at 3 d. Increase in PCNA ⁺ cells and decrease in TUNEL ⁺ cells and BAX.	[134]

(continued on next page)

Table 2. (continued)

Bioactive compound/gene	Delivery mechanism	Recipient(s)	Disease (model)	Details	Key findings	Refs
hAQP-1	Adenovirus	Wistar rats	RIX	5×10^9 pfu/gland, 3 mo post-IR (17.5 Gy) or 4 mo post-IR (21 Gy), delivered intraductally to SMG.	Up to fivefold increase of AQP1 in acinar and ductal cells of rat SMG. Modest (~30%, nonsignificant) increase in saliva production in non-IR rats, significant (two- or threefold) increase in saliva production in IR rats exposed to 21 Gy and 17.5 Gy, respectively.	[128]
hAQP-1	Adenovirus	Rhesus monkeys	RIX	2×10^9 or 1×10^8 pfu/gland, 19 wk post-IR (10 Gy), delivered intraductally to the PG.	Lower expression of transgene than in rat studies [128]. Immunostaining of hAQP-1 visible in some vasculature, acini, and ducts. Improvements in saliva flow range from modest (20%–50%) to higher (≥ 2 -fold). Control virus results in inconsistent (65% increase by day 3, return to baseline at day 7, and 25%–50% increase at day 14) changes in saliva production.	[130]
hAQP-1	Adenovirus	Miniature pigs	RIX	10^8 or 10^9 pfu/gland, 17 wk post-IR (20 Gy), delivered by retrograde infusion to the PG.	Incorporation of hAQP-11 in to pig ductal cells. Increase in saliva production 3 d and 16 wk post-IR ($81\% \pm 18\%$ of pre-IR values). Rapid return to pre-IR K^+ levels with hAQP-1. Increased white blood cell count in pigs administered adenoviral vectors (regardless of whether control or hAQP-1).	[129]
hAQP-1	Adenovirus	Humans	RIX	Safety testing: 4.8×10^7 to 5.8×10^9 vector particles per gland delivered to previously irradiated PG. 11 subjects.	No deaths, dose-limiting toxicities, or serious adverse events as result of vector delivery. Subjective improvement in oral dryness in 5 of 11 patients, no change or worsening in 4 of 11 patients. Elevated salivary flow 3–4.7 y after treatment in 5 of 11 patients, with improved symptoms for ~2–3 y. Three of 11 patients reported dry mouth as bad as at start of trial by 3-y follow-up.	[135,136]
hAQP-1	Adenovirus	Humans	SS	No details	Results not yet published	Not published

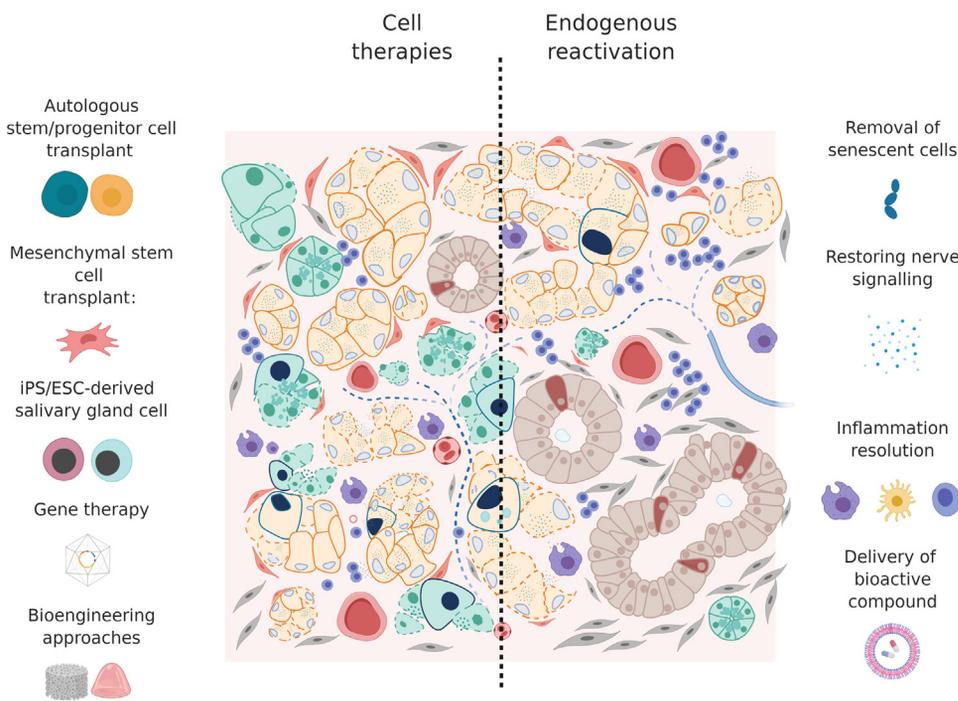
AQP1, aquaporin 1; AQP5, aquaporin 5; BrdU, bromodeoxyuridine; γ H2AX, histone family member X; hKGF, human keratinocyte growth factor; hrbFGF, human recombinant fibroblast growth factor; IGF-1, insulin-like growth factor 1; IR, irradiation; IV, intravenously; mNKCC1, murine Na-K-Cl cotransporter; mPkc δ , murine protein kinase C δ ; PCNA, proliferating cell nuclear antigen; PG, parotid gland; RIX, radiation-induced xerostomia; SC, subcutaneously; SCC, squamous cell carcinoma; SG, salivary gland; SMG, submandibular gland; SS, Sjögren's syndrome; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labelling.

environment following injury or disease influences exogenous or endogenous attempts to regenerate tissue. Senescent cells and accompanying senescence-associated secretory phenotype can negatively influence neighbouring cells and likely provide an environment that is not conducive to regeneration. One could speculate that a future approach whereby senescent cells were eliminated, similar to studies conducted in the ageing heart [139], could provide a more hospitable environment for SG regeneration. Similarly, modulating inflammation to be proreparative [140] may sufficiently alter the cellular environment to promote regeneration.

Furthermore, ESCs and iPSCs are an attractive tool for regenerative medicine. Secretory-like cells can be derived from murine ESCs (mESCs) [141], and the first successful murine transplant of lab-grown **orthotopically** functional SG derived from mESCs was published in 2018 [142], signifying that there is an effort to explore alternative sources of SG stem cells for regenerative medicine. At the time of writing this review, there has been just one published study where iPSCs have been used for SG regeneration, where the authors concluded that engraftment of mESCs was benefitted following coculture with iPSCs, an effect they attributed to an improvement in the niche but not to direct engraftment of iPSCs themselves into salivary tissue [143]. However, there are significant ethical issues with the use of ESCs, and substantial challenges remain for iPSC use, namely reprogramming efficiency and unpredictable outcomes, precluding their widespread adoption at present. Overall, a better understanding of all the contributing factors that lead to regeneration will be advantageous for both exogenous and endogenous future therapies.

Key Figure

Schematic Representation of Current and Future Approaches for Salivary Gland Regeneration



Trends in Molecular Medicine

Figure 4. Regenerative approaches for salivary gland dysfunction and xerostomia are broadly based on either exogenous delivery of cells or genes, or endogenous reactivation of resident cells or tissue components. Exogenous delivery of cellular therapies to regenerate injured tissue, as presented on the left half of the image, includes isolation and autologous transplant of salivary gland stem/progenitor cells, mesenchymal stem cell transplant, transplant of iPSC- or ESC-derived salivary gland cells, and gene therapy, coupled with bioengineering approaches to deliver cells/genes. In contrast, endogenous reactivation of components of the niche to promote regeneration, as presented on the right half of the image, includes removal of senescent cells, restoration of nerve signalling, resolving inflammation, and the delivery of bioactive compounds, and its aim is to establish a proregenerative tissue environment critical for regeneration of the damaged tissue during injury, disease, and ageing. (Figure drawn using BioRender.)

Clinician's Corner

Patients experiencing hyposalivation may experience accelerated tooth decay and dental caries, oral mucositis and/or candidiasis, recurrent **sialadenitis**, **dysgeusia**, **dysphagia**, and a fissured tongue.

Radiotherapy remains the predominant curative treatment for head and neck cancer and, while largely effective at targeting the tumour, more than 75% of patients experience salivary gland dysfunction and xerostomia following radiotherapy. In addition, one of the hallmark symptoms of the autoimmune disease Sjögren's syndrome is immune cell infiltration into glandular tissues, resulting in salivary gland destruction and hyposalivation. There is currently no restorative cure for hyposalivation, and patients rely on treatments that manage the symptoms but do not target the underlying cause.

Potential approaches to regenerate salivary gland tissue involve isolating, expanding, and transplanting adult tissue-resident stem cells that are able to give rise to new salivary tissue, restoring signals of the stem cell niche to promote endogenous regeneration of the glands, delivering essential components of the gland by gene therapy, and bioengineering approaches to deliver cells or bioactive particles within a supportive scaffold.

In the future, it may be possible to take a precision medicine approach whereby skin cells are taken from the patient and dedifferentiated into stem cells (iPSCs), which can then be transplanted back into the patient to regenerate a functional gland and permanently restore salivary gland function.

Concluding Remarks

Whilst enormous progress in the effort to develop a regenerative strategy for xerostomia has been made in recent years (Figure 4, Key Figure), there remains a clinical bottleneck, compounded by differences in human SGs and those of animal models used, the differences in pathological destruction of the SGs, and the vast range of confounding clinical factors in human patients (see Outstanding Questions). While rodent and human SGs share many similarities, practical considerations when translating studies from mouse to human should be taken into consideration. For example, cell dissociation protocols that have been optimised for murine tissue are not necessarily ideal for dissociating cells from human-derived tissue, especially biopsies from older patients, given the extent of connective tissue, possible fibrosis, and sensitivity of the cells (reviewed in [144]). In addition, while numerous studies have shown the potential of SGSCs derived from mouse SMGs to rescue murine SG function following IR, to date only one published study has demonstrated that human-derived SGSCs have the same potential [83]. Of note, this study relied on an IR dose of 5 Gy, and results are yet to be produced following a dose consistent with others in the field showing successful murine SGSC transplants (10–15 Gy). Thus, consistency in injury models when comparing the potential of murine and human cells is essential to be comparative.

Patients with xerostomia often present with contributing factors that may influence their recovery and treatment and their ability to tolerate a regenerative therapy. For example, SS is characterised by inflammatory cell- and cytokine-mediated tissue destruction and eventual stem cell exhaustion, while IR-induced xerostomia is characterised by cellular senescence and fibrosis. Thus, a **precision medicine** approach whereby treatment is individualised to each patient, dependent on age, cause of tissue damage, and contributing systemic factors, and which uses an autologous cell transplant or factor delivery to the injured tissue, provides an attractive therapeutic direction. However, with present knowledge and medical technology, this will rely on approaches that range from currently achievable to not available: (i) delivering a factor to the gland that can significantly alter the function and/or regenerative ability; (ii) successfully isolating, maintaining, and expanding stem/progenitor cells from patient SGs prior to RT; or (iii) generating large numbers of stem cells via reprogramming of iPSCs. Furthermore, whether the injured human SG can regenerate itself under adverse conditions remains unknown. Murine studies have provided evidence that, under severe injury, tissue-resident progenitor cells can undergo transdifferentiation [28,95,97]; however, whether this can occur in human SGs remains unanswered, but a report that human hepatocytes can transdifferentiate into biliary cells when transplanted into a mouse liver [145] provides encouraging evidence that cells of human origin can undergo transdifferentiation *in vivo* in response to injury. In conclusion, future studies demonstrating success of the above approaches in human trials will be pivotal in working toward a permanent cure for xerostomia.

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Outstanding Questions

Do common salivary gland stem cell markers exist in both rodents and humans? Mice are commonly used to model human diseases in regenerative medicine research. A number of markers have been proposed for the isolation of murine SGSCs and have been demonstrated to be efficacious at improving salivary function in recipient mice, but these same markers are often either lacking in human SG or do not mark cells with stem cell properties. As such, developing methods to be able to better translate murine studies to be human-relevant will be of upmost importance for future studies.

Will stem cells transplants ever be successful if the recipient gland environment is unfavourable? Often, damaged glandular tissue is fibrotic or inflamed or contains senescent cells. Studies in other organs have demonstrated that the host environment plays a crucial role in the success of stem cell transplants, regardless of the state of the cells being transplanted. In order for future regenerative approaches to be successful, this must be taken into consideration, and strategies to provide a more hospitable environment will be essential.

Can the efficiency of iPSC reprogramming be improved enough to make iPSC stem cell generation a viable and cost-effective precision medicine approach? In theory, iPSCs provide a noninvasive and abundant source of stem cells. However, the current challenges in iPSC use include cells retaining some epigenetic memory of the tissue from which they were derived, the very low efficiency in reprogramming, genetic instability, and the risk of tumourigenicity. Better characterisation of iPSCs will likely lead to significant pipeline improvements that could enable patient-specific stem cell generation in the future.

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