Mouth-watering results: clinical need, current approaches and future directions for salivary gland regeneration

Mouth-watering results: clinical need, current approaches and future directions for salivary gland regeneration

Cecilia Rocchi and Elaine Emmerson

The MRC Centre for Regenerative Medicine, The University of Edinburgh, Edinburgh BioQuarter, 5 Little France Drive, Edinburgh, EH16 4UU.

Keywords: salivary glands, xerostomia, regeneration, stem cells, bioengineering, gene therapy, transdifferentiation

Abstract

Permanent damage to the salivary glands and resulting hyposalivation and xerostomia has a substantial impact on patient health, quality of life and healthcare costs. At present patients rely on lifelong treatments that alleviate the symptoms but currently, 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 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 function also hinders oral functions including speaking and taste, due to insufficient lubrication of the oral mucosa [1] (**Clinician's Corner**). In addition, saliva has essential anti-microbial properties and, as such, the salivary glands 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 salivary glands, located around the jaw: the parotid gland (PG), submandibular gland (SMG) and sublingual gland (SLG). The major salivary glands produce >90% of the total saliva which is secreted in to the oral cavity, with the remaining <10% being produced by the minor labial salivary glands, found in the lips [reviewed in 2]. In humans, the largest of the major salivary glands, 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 salivary glands 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] (**Figure 1A, Figure 2A**).

Salivary dysfunction and hyposalivation often occurs as a result of Sjögren's Syndrome , radiotherapy, cancer, or aging. Permanent destruction of the secretory **acini** of the salivary gland 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 discuss the clinical need for salivary gland 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 Sjögren's Syndrome (SS), following immune-modulated destruction of the salivary glands over many years [12]. Excessive inflammatory cell infiltration and subsequent elevated cytokine production and tissue proteolysis destroys the saliva-synthesising acinar cells in SS patients [13] (**Figure 2B**), leading to salivary dysfunction. Destruction of the secretory acini severely impacts oral health and quality of life of patients and they are at significantly higher risk of developing oral cavities (due to the loss of the anti-microbial 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 300,000 deaths. Therapeutic radiation (IR) remains a life-saving treatment for cancers of the head and neck. However, the salivary glands often also lie within the field of therapeutic radiation, and thus are inadvertently irradiated along with the tumour [15]. **Fractionated radiation** (2Gy, five days a week, over 5-7 weeks) is a commonly used therapeutic intervention, given as such to reduce the damage to non-malignant tissue, since the tumour and normal tissue react differently to such a dose [16]. Specifically, normal tissue can repair DNA damage induced by 2Gy IR better than malignant tissue [17]. Despite the fact that salivary glands have a relatively slow turnover rate, when compared to 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 using 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 with the acute salivary dysfunction observed and with other studies. Such reports demonstrate 1) elevation of apoptosis markers [24]; 2) loss of acinar cells and a decline in salivary flow rate [24-26]; 3) decrease in amylase secretion [24, 25]; 4) plateau; 5) 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 salivary glands, 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 salivary function and dry mouth. In addition, a common side-effect (~25%) of parotidectomy is **facial palsy**, due to transient or permanent post-operative facial nerve damage [33]. Given the recently documented role of nerve signalling in salivary gland homeostasis and regeneration [24, 34], post-operative nerve damage may have a more significant effect on salivary gland function following tumour resection than previously thought.

Ageing

As with many organs the salivary glands 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 TUNEL-positive apoptotic cells increase in the SMGs with increasing age, suggesting that a contributing factor in a decline in salivary 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 National Institutes for 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 salivary gland function), aimed towards the restoration of the damaged salivary gland, reporting the highest activity in the USA and Europe (**Figure 3**). Of 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 FDA approval mainly comprise of stimulant medications or **secretagogues**, and salivary substitutes or artificial salivas (**Figure 3**), as described below, that provide temporary release from the discomfort caused by the symptoms, but at present, no permanent solution to the problem, the irreversible damage to the functional acinar cells of the gland, exists.

Saliva substitutes

Saliva substitutes or artificial saliva are often prescribed as a temporary relief from xerostomia and in general contain as thickening agents and to provide 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 phage [43], and dysregulation of the oral microbiota may contribute to exacerbating the severity of the side effects associated with radiation, 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 (VAS) 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 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 radiation therapy patient-derived human salivary gland 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 sialogogues approved by the United States Food and Drug Administration (FDA) and the National Institute of Clinical Excellence (NICE) 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 vasodilatation, 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 has demonstrated a significant decrease in xerostomia severity with use, the frequency of xerostomia symptoms remained unchanged [55].

78
79 **Potential approaches in pre-clinical/clinical testing**
80
81 *Stem cell transplants*
82
83 A potential regenerative approach, not only for salivary glands, but for organs as a whole, is through the
84 use of stem cell therapy [56]. Stem cell therapy-based approaches account for 11.9% of the clinical
85 interventions for xerostomia (**Figure 3**). While the progress of stem cell clinical trials for xerostomia is
86 encouraging, given that the majority are in early phase 1 or 2, these approaches rely solely on the use of
87 mesenchymal stem/stromal cells (MSCs) [reviewed in 57].
88
89 *Mesenchymal stem/stromal cells*
90
91 MSCs are an attractive source of stem cells due to their relative abundance and the ability to harvest in a
92 non-invasive manner. The majority of *in vivo* MSC salivary transplant studies that have been published
93 harvest MSCs from bone marrow, adipose tissue or umbilical cord blood [58-69] or involve isolating
94 peripheral blood mononuclear cells (PBMNCs) and differentiating into effectively conditioned PBMNCs (E-
95 MNCs) [70] (Table 1). However, MSCs have limitations that precludes their widespread adoption as a stem
96 cell therapy, with the primary concern being of safety. There is yet to be any report of the long-term safety
97 of MSCs and because of their **immune privilege**, they have the potential to metastasise to other tissues
98 or organs. In addition, their use has been associated with tissue fibrosis and their heterogenous nature
99 and donor-dependent efficacy poses a significant hurdle [71]. As such the identification of adult tissue-
100 resident stem cells that can regenerate functional salivary tissue will be of considerable benefit to the field.
101
102 *Enrichment of salivary gland-derived stem cells*
103
104 Identification of cells in the adult salivary gland that exhibit stem cell characteristics and are able to rescue
105 irradiation-induced hyposalivation when transplanted *in vivo* is encouraging evidence of the existence of
106 adult tissue-resident stem/progenitor cells. While, thus far, the isolation of murine salivary gland stem cells
107 (SGSCs) has been mainly based on markers of adult stem cells identified in other tissues, transplantation
108 of these cells has provided a proof-of-concept that adult SGSCs could be a viable approach to treat
109 xerostomia. The *in vivo* functionality of murine SGSCs was first demonstrated with selected primary
110 sphere-derived KIT+ cells [72]. In brief, cell suspensions were prepared by mechanical and enzymatic
111 digestion of donor mouse SMG in collagenase II, hyaluronidase and $CaCl_2$ and incubated in supplemented
112 growth medium to generate spheres. Female recipient mice that had been previously irradiated with 15
113 Gy were transplanted with 60,000-90,000 SGSC spheres after 3 days in culture via intraglandular injection,
114 30 days post-IR. While transplant of these cells significantly improved saliva production in 42% (5/12
115 animals) compared to irradiated non-transplanted mice, later studies showed that selection and

enrichment of CD24+CD29+ salivary gland-derived cells improved regeneration of the acinar compartment and allowed up to 70% rescue of normal salivary gland production compare to non-irradiated control [73]. Furthermore, the development of a salivary gland-derived **organoid** culture not only allowed for expansion of rare SGSCs [73-75], but also allowed the investigation and characterisation of key niche signalling components necessary for salivary gland homeostasis and regeneration [76-79]. WNT stimulation of salivary gland organoid cultures results in the expansion of the salivary gland 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 salivary gland 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 salivary gland stem cells has been demonstrated, and 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

Radiation 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 head and neck cancer patients at diagnosis (63-65 years of age), together with the natural decline of adult tissue stem cells from aged SG [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 salivary glands 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 re-activate and expand the resident stem/progenitor cell pool.

Moreover, while murine studies have provided compelling evidence that cells within the salivary glands have regenerative potential, further experiments are required to establish if human SGSCs can regenerate functional tissue in human xerostomia patients and provide long-term salivary rescue. The majority of murine studies discussed above have utilised KIT, CD24 and CD29 as markers of SGSCs. Although these markers appear to be present in human salivary gland [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, in order to develop an autologous stem cell isolation and transplantation protocol will require cells be stored for a period of time following isolation, before the post-IR gland is ready for transplantation. The cryopreservation and storage of rat SGSCs for 3 years provides 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 transplants 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 duct cells [24, 88], KRT14+ cells divide asymmetrically to give rise to larger granular ducts [86, 88] and a population of overlapping but non-identical KRT5+ and AXIN2+ cells produce intercalated and excretory duct 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 has 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 (NKCC1) and inositol 1,4,5-triphosphate receptor 3 (IP3R3) [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 transdifferentation 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 MMG luminal duct (MCF10A) cell lines induces expression of *Kit,* and when cultured in 3D conditions 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 non-autonomous mechanisms control cellular plasticity and signals from the stem cell niche, such as WNT signals in the intestine [98], may control de-differentiation 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 trans-differentiation 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, 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 salivary gland stem/progenitor cell niche comprises of multiple signals during homeostasis, including the cellular components such as the nerves, blood vessels and surrounding mesenchymal cells; secreted factors; inflammatory cells, extracellular matrix (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 salivary gland regeneration.

While the regulation of saliva secretion by neuronal signals is well documented [8], the role that nerve signals play on salivary gland 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 which test the effect of novel muscarinic mimetics to drive *in vivo* salivary gland 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 salivary gland development and regeneration [reviewed in 102]. Glial cell-derived neurotrophic factor (GDNF) is highly expressed in murine salivary gland stem cells 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 salivary gland development [106] and its expression is upregulated in the ducts of the adult murine SMG during regeneration, following **duct ligation** injury [107]. Of importance to regenerative strategies, the transient activation of WNT in a murine model prevents radiation-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 salivary gland 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 irradiation-damaged SG likely plays a role in the 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 extracellular matrix (ECM) stiffness in multiple tissues during development, regeneration and tumourigenesis. Moreover, murine salivary gland 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 salivary dysfunction is through the use of bioengineering. Isolated salivary gland cells cultured with the 3T3 cell line, were able to engraft in to 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 salivary gland 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 (EGF) and insulin-like growth factor (IFG-1), produced mature-looking structures capable of amylase production [120], while human salivary cells cultured in a 3D system express differentiation markers aquaporin-5 (AQP5), occludin and amylase, and polarity markers ZO-1 and claudin-1 [121]. Moreover, decellularised rat SMG 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 salivary gland is a promising therapeutic option. Retroductal delivery, by injecting in to the major duct, has been previously undertaken to administer a multitude of reagents, including growth factors, primary cells, adenoviral vectors, cytokines, and antioxidant compounds [23, 123-133]. Donor rat salivary gland cells, also administered through the major duct, are reported to be able to incorporate in to the salivary gland 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 normal homeostatic gland was over 50 times more than in injured gland, suggesting that bFGF can promote tissue repair in the murine SMG [124]. Similarly, mice administered with IGF-1 exhibited improved stimulated salivary flow and amylase production 30 days post-irradiation, activation of Akt and a complete rescue of salivary function to a level comparable with that of non-irradiated 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 radiation-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 salivary gland epithelia is often considered one of the hallmarks of salivary dysfunction and recent advances in the field have aimed to restore permeability in an attempt to increase salivary production. In 1997, the first salivary gland 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 radiation, led to a two- to threefold increase in salivary secretion, compared to that of a control virus, suggesting that hAQP1 delivery may be a promising therapeutic approach to restore salivary function after radiation injury. Follow-on studies demonstrated similar efficacy in miniature pigs [129], while results in rhesus monkeys were less consistent (2/4 showed improved salivary flow) [130]. Similarly, targeted increase of gland permeability by AQP1 AAV2 gene therapy in a murine model of Sjögren's Syndrome (SS) improved salivary flow and reduced inflammation, specifically T cell infiltrate [131], implicating the positive effect that gene therapy may have to treat patients with SS. At the date of publishing this review, both preclinical work investigating AAV-AQP1 as a means to restore salivary gland function in SS patients and a phase 1 dose escalation clinical study to treat salivary dysfunction in patients with grade 2 or 3 radiation-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 radiation therapy 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, well tolerated and improved PG salivary flow in a subset of patients [135]. Moreover, these 5 patients experienced elevated salivary flow 3-4.7 years after treatment, with improved symptoms for ~2-3 years [136]. Of importance, many adeno-associated virus (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 SS patients. Neutralisation of B cell-activating factor (BAFF) and proliferation-inducing ligand (APRIL) 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 radiation-induced SMG dysfunction, and salivary flow, measured following pilocarpine stimulation, was similar in mice administered the AdLTR(2)EF1α-hKGF vector compared with non-irradiated controls [123]. Table 2 outlines the details of published studies where gene therapy has been used as a therapeutic measure for SG dysfunction. However, while providing encouraging results, gene therapy also has its challenges, including the risk of the recipient mounting an immune response, 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 there still remain some hurdles. One aspect that has received little attention is how the cellular environment following injury or disease influences exogenous or endogenous attempts to regenerate tissue. Senescent cells and accompanying senescence-associated secretory phenotype (SASP) can negatively influence neighbouring cells and likely provides an environment which 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 salivary gland regeneration. Similarly, modulating inflammation to be pro-reparative [140] may sufficiently alter the cellular environment to promote regeneration.

Furthermore, embryonic stem cells (ESCs) and **induced pluripotent stem cells (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 salivary gland derived from mESCs was published in 2018 [142], signifying that there is an effort toward exploring 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 utilised for salivary gland regeneration, where the authors concluded that engraftment of mESCs was benefitted following co-culture with iPSCs, an effect they contributed to an improvement in the niche, but not direct engraftment of iPSCs themselves in to 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.

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 mouse 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 SMG to rescue murine salivary gland 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.

Xerostomia patients often present with contributing factors that may influence their recovery, treatment and their ability to tolerate a regenerative therapy. For example, Sjögren's Syndrome is characterised by inflammatory cell- and cytokine-mediated tissue destruction and eventual stem cell exhaustion, while radiation-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 1) delivering a factor to the gland that can significantly alter the function and/or regenerative ability; 2) successfully isolating, maintaining and expanding stem/progenitor cells from patient salivary gland prior to radiation therapy; or 3) generating large numbers of stem cells via reprogramming of iPSCs, approaches that range from currently achievable to not available. Furthermore, whether the injured human salivary gland 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 salivary glands remains unanswered, but a report that human hepatocytes can transdifferentiate into biliary cells when transplanted into a mouse liver [145] is 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.

Clinician's Corner

- Patients experiencing hyposalivation may experience accelerated tooth decay and dental caries, oral mucositis and/or candidiasis, recurrent **sialadentitis**, **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 dysfunction and xerostomia following radiotherapy. In addition, one of the hallmark symptoms of the autoimmune disease Sjögren's Syndrome is immune cell infiltration in to 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 de-differentiated in to stem cells (iPSCs), which can then be transplanted back in to the patient to regenerate functional gland and permanently restore salivary gland function.

Table 1. Stem cell transplants as a salivary gland regenerative approach

Donor	Stem cell	Recipient	Disease (model)	Details	Key Results	Ref
C57/BL6 mice	BMdMSCs	C57/BL6 mice	RIX	$1x10^5$ MSCs injected in to each SMG, 24h post-IR (15Gy)	Cell integration after 4 weeks. Significant increase in salivary flow, increased functional acinar cells, reduced apoptosis and increased vasculature.	[58]
C57BL/6-Tg(CAG-EGFP) mice	AdMSCs	C57/BL6 mice	RIX	$1x10^5$ MSCs injected in to each SMG, 10wks post-IR (10Gy)	Cell integration in to ductal and endothelial cells. Improved salivary flow and increased angiogenesis.	[59]
C57/BL6 mice	BMdMSCs	NOD.SCID-*Prkc*^{SCID} mice	RIX	$1x10^5$ MSCs injected in to each SMG, 11d post-IR (15Gy)	Significant increase in salivary production, increased body weight and gland weight.	[60]
C57BL/6-TgH/BALB/c mice	BMdMSCs	NOD mice	SSLMM	$1x10^5$ MSCs injected IV, at 6 or 16 wks of age	Improved salivary flow, reduced lymphocytic infiltration, reduced T and B cells and increased T-regs.	[61]
Balb/c or C57BL/6-gfp	BMdMSCs	NOD/Ltj mice	SSLMM	$1x10^5$ MSCs injected IV, at 6 or 16 wks of age	Cell integration. Reduced inflammation, T cell polarisation toward T-reg 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 in to each SMG, 3d post-IR (12Gy)	Cell engraftment in to 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 (4,000-7,000), KIT^+ cells (300-1,000) or KIT^- cells (10,000-90,000) injected in to each SMG, 30d post-IR (15Gy)	Cell integration and formation of ductal structures at the injection site and mucin+ cells throughout the gland (after 90 days). Increased cell proliferation, increased acinar cells and significantly improved saliva production.	[72]
C57BL/6 mice	SGSCs	C57BL/6 mice	RIX	400 KIT^+ cells, 1,000 KIT^+CD24^+ cells or 400 $KIT^+CD24^+CD49f^+$ cells injected in to	Improved salivary production (90 days post-transplant), increased expression of ductal markers, rescued	[73]

				each SMG, 30d post-IR (15Gy)	vascularisation and reduced fibrosis.	
C57BL/6 mice	SGSCs	C57BL/6 mice	RIX	5,000 $CD133^+$, 150 KIT^+CD49f^+ or 5000 $CD24^+CD29^+$ cells injected in to each SMG, 30d post-IR (15Gy)	Increased saliva production (120 days post-transplant), with all cell transplants compared to control (however, no significance reported). Most substantial improvement with $CD133^+$ or $CD24^+CD29^+$ cells.	[74]
C57BL/6-Tg(CAG-EGFP)1Osb/J and B 6.Cg-Tg(CAG-DsRed*MST)1Nagy/J mice	SGSCs	C57BL/6 mice	RIX	5000 cells injected in to each SMG, 30d post-IR (15Gy)	Donor cell (GFP^+) engraftment. Improved saliva production (60, 90 and 120 days post-transplant; 46% ± 2.11%). Reappearance of functional acinar cells (visualised by histology and AQP5 immunostaining). No sign of cell transformation or tumourigenesis.	[75]
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, 1,000 or 10,000 cells injected in to each SMG, 28d post-IR (15Gy)	Donor cell (RPF^+) engraftment. Improved saliva production (120 days 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, 5,000 or 50,000 salisphere cells injected in to each SMG, 30d post-IR (5Gy)	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	$1x10^6$ MSCs injected IV (tail vein), immediately after IR (15Gy) and every wk therafter for 3 wks	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	$1x10^6$ MSCs injected SC to each	Improved salivary flow, improved angiogenesis,	[66]

				SMG, immediately after IR (18Gy)	elevated expression of VEGF reduced apoptosis and reduced fibrosis.	
Humans	UCdMSCs	Humans	SS	One dose of $1x10^6$ MSCs per kg bodyweight injected IV	Improvements in Sjögren's Syndrome Disease Activity Index (SSDAI) scores and improved salivary flow rate (at 2 wks and 1 month post-transplant). Abolished production of anti-SSA/Ro in serum.	[62]
Humans	AdMSCs	Humans	RIX	2.8×10^6 MSCs injected in to each SMG, at least 2 years post-radiotherapy/chemo-radiotherapy	Improvements in salivary flow rate (at 1 and 4 months post-transplant), amelioration of the symptoms of xerostomia. Increased serous cells and reduced adipose and connective tissues.	[67, 69]

BMdMSCs: bone marrow-derived mesenchymal stem cells; AdMSCs: adipose-derived mesenchymal stem cells; SGSCs: salivary gland stem cells; UCdMSCs: Umbilical cord-derived mesenchymal stem cells; PBMNCs: peripheral blood mononuclear cells; E-MNCs: effectively conditioned mononuclear cells; *RIX: radiation-induced xerostomia; RIIX: radioiodine-induced xerostomia; SSLMM: Sjögren's Syndrome-like mouse model; SS: Sjögren's Syndrome.*

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	Ref
Rat acinar cell "slurry"	Primary cell delivery	Sprague-Dawley rats	None	250 to 300 µl cell slurry delivered via SMG ductal cannulation.	Donor cells persisting after 21 days post-transplant. However, claims of cytodifferentation 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 15Gy or fractionated dose of 6Gy for 5 days), delivered intraductally to SMG.	Secreted hKGF detectable in murine SMGs after 9 weeks. Improved salivary flow rate and maintained body weight after 9 weeks. Increased cycling ($BrDU^+$) cells, no difference in $AQP5^+$ cells, non-significant 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 wks post-ligation, delivered via	SMG weight increase, recovery of acini, increase in $PCNA^+$ cells and reduction in fibrosis.	[124]

				ductal cannulation to SMG.		
mNKCC1 or mPkcδ	siRNA delivery	BALB/c/cByJ mice	RIX	4 µg siRNA/gland, pre-IR (10Gy), 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 Pkcδ blocked the upregulation of Pkcδ mRNA usually seen following IR, coupled with an improvement in morphology and increased $AQP5^+$ acinar cells at 90 days and reduced apoptosis (by 70%) at 48 hrs after IR. Rescue of saliva secretion (80±26% of control).	[132]
mIGF-1	Growth factor delivery	FVB mice	RIX	5 µg recombinant IGF-1, days 4-8 post-IR (5Gy or 2Gy per day for 5 days), 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 µg IL-6, HIL-6 or anti-IL-6 administered 2 wks pre-IR (13 Gy (single fraction) or 28 Gy in 5 daily fractions of 5.6 Gy), delivered IV.	No difference in induction of γH2AX (DNA damage) up to 48 hrs, but substantially decreased 48-72 hrs with IL-6 pre-treatment compared to control. Up to 3-fold increase in saliva production and a reduction of $p21^+$ acinar cells with IL-6 or HIL-6 pre-treatment at 8 wks post-IR. Rescued salivary production 8 wks post-IR with anti-IL-6.	[23]
Histamine	Compound delivery	Sprague-Dawley rats	IR	Daily histamine injections (delivered SC, 0.1 mg/kg) starting 24 hrs before IR (5 Gy, whole body).	Rescue of saliva production compared to IR alone at 3 days. Increase in $PCNA^+$ cells and decrease in $TUNEL^+$ cells and BAX.	[134]

hAQP-1	Adenovirus	Wistar rats	RIX	5×10^9 pfu/gland, 3 months post-IR (17.5 Gy) or 4 months post-IR (21 Gy), delivered intraductally to SMG.	Up to 5-fold increase of AQP1 in acinar and ductal cells of rat SMG. Modest (~30%, non-significant) increase in saliva production in non-IR rats, significant (2 or 3-fold) increase in saliva production in IR rats exposed to 21Gy and 17.5 Gy, respectively.	[128]
hAQP-1	Adenovirus	Rhesus monkeys	RIX	2×10^9 or 1×10^8 pfu/gland, 19 wks post-IR (10Gy), 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 ($\geq$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 wks 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 days and 16 wks post-IR (81±18% of pre-IR values). Rapid return to pre-IR K^+ levels with hAQP-1. Increased white blood cell count in pigs administered with 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 5/11 of patients, no change or worsening in 4/11 of patients. Elevated salivary flow 3-4.7 years after treatment in 5/11 patients, with improved symptoms for ~2-3 years. 3/11 patients reported dry	[135, 136]

					mouth as bad as start of trial by 3 year follow-up.	
hAQP-1	Adenovirus	Humans	SS	No details	Results not yet published	Not published

RIX: radiation-induced xerostomia; IR: whole body irradiation; SS: Sjögren's Syndrome.

Figure Legends

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, 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 (ECM); components of metabolism and physical cues. Figure drawn using BioRender.

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. Homeostasis of the healthy salivary gland epithelium **(A)** 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. During Sjögren's Syndrome **(B)**, 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. Therapeutic radiation **(C)** 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, leads to irreversible disruption of the glandular niche. During ageing **(D)** 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.

Figure 3. Pre-clinical and clinical approaches for the treatment of xerostomia. At the time of writing this review 301 studies, including both observational and interventional, were registered in the NIH 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 in 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, at 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.

Figure 4, Key Figure. Schematic representation of current and future approaches for salivary gland regeneration. 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 transplantation of salivary gland stem/progenitor cells, mesenchymal stem cell transplantation, transplantation 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 nerve signalling, resolving inflammation and the delivery of bioactive compounds, and aims to establish a pro-regenerative tissue environment critical for regeneration of the damaged tissue during injury, disease and ageing. Figure drawn using BioRender.

Acknowledgements

The authors would like to thank Ella Mercer for critically reading the manuscript.

Glossary

Acini: the functional saliva-producing unit of the salivary gland

Duct ligation: a technique where 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 in to a number of sessions and given over several weeks. Performed in order to minimise side effects by allowing normal tissues to recover in between sessions.

Hyposalivation: a substantial reduction in saliva production from all salivary glands or one gland in particular

Immune privilege: cells or tissue that do not elicit an inflammatory immune response from the host

Induced pluripotent stem cells (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 3-dimensional 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 where 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

Salisphere: spheroid salivary gland organoid

Secretagogues: a substance that promotes secretion and salivation

Sialadentitis: infection of the salivary gland

Xerostomia: dry mouth resulting from a change in the composition of saliva or reduced/absent saliva flow

References

1. Tsai, W.L. et al. (2014) Impact of late toxicities on quality of life for survivors of nasopharyngeal carcinoma. BMC Cancer 14, 856.
2. Miletich, I. (2010) Introduction to Salivary Glands: Structure, Function and Embryonic Development. In Salivary Glands. Development, Adaptations and Disease (Tucker, A.S. and Miletich, I. eds), pp. 1-20, Front Oral Biol. Basel, Karger.
3. Emmerson, E. and Knox, S.M. (2018) Salivary gland stem cells: A review of development, regeneration and cancer. Genesis 56 (5), e23211.
4. Martinez-Madrigal, F. and Micheau, C. (1989) Histology of the major salivary glands. Am J Surg Pathol 13 (10), 879-99.
5. Amano, O. et al. (2012) Anatomy and histology of rodent and human major salivary glands: -overview of the Japan salivary gland society-sponsored workshop. Acta Histochem Cytochem 45 (5), 241-50.
6. Segawa, A. et al. (1995) [Function of myoepithelial cells in salivary secretion: reevaluation of the expulsion theory]. Kaibogaku Zasshi 70 (4), 330-7.
7. Kwon, H.R. et al. (2017) Endothelial cell regulation of salivary gland epithelial patterning. Development 144 (2), 211-220.
8. Proctor, G.B. and Carpenter, G.H. (2007) Regulation of salivary gland function by autonomic nerves. Auton Neurosci 133 (1), 3-18.
9. Togarrati, P.P. et al. (2018) CD29 is highly expressed on epithelial, myoepithelial, and mesenchymal stromal cells of human salivary glands. Oral Dis 24 (4), 561-572.
10. Oudghiri, M. et al. (1986) The cellular basis of salivary immunity in the mouse: incidence and distribution of B cells, T cells and macrophages in single-cell suspensions of the major salivary glands. Eur J Immunol 16 (3), 281-5.
11. Jensen, S.B. et al. (2010) A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: prevalence, severity and impact on quality of life. Support Care Cancer 18 (8), 1039-60.
12. Stewart, C.M. et al. (2008) Salivary dysfunction and quality of life in Sjogren syndrome: a critical oral-systemic connection. J Am Dent Assoc 139 (3), 291-9; quiz 358-9.
13. Perez, P. et al. (2005) Increased acinar damage of salivary glands of patients with Sjogren's syndrome is paralleled by simultaneous imbalance of matrix metalloproteinase 3/tissue inhibitor of metalloproteinases 1 and matrix metalloproteinase 9/tissue inhibitor of metalloproteinases 1 ratios. Arthritis Rheum 52 (9), 2751-60.
14. Pringle, S. et al. (2019) Salivary Gland Stem Cells Age Prematurely in Primary Sjogren's Syndrome. Arthritis Rheumatol 71 (1), 133-142.
15. Ortholan, C. et al. (2010) [Normal tissue tolerance to external beam radiation therapy: salivary glands]. Cancer Radiother 14 (4-5), 290-4.
16. Bernier, J. and Horiot, J.C. (2012) Altered-fractionated radiotherapy in locally advanced head and neck cancer. Curr Opin Oncol 24 (3), 223-8.
17. Vissink, A. et al. (2003) Oral sequelae of head and neck radiotherapy. Crit Rev Oral Biol Med 14 (3), 199-212.
18. Vissink, A. et al. (2010) Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys 78 (4), 983-91.
19. Grundmann, O. et al. (2009) Sensitivity of salivary glands to radiation: from animal models to therapies. J Dent Res 88 (10), 894-903.
20. Lombardini, E.D. et al. (2013) Chapter 44 - Radiation and Other Physical Agents. In Haschek and Rousseaux's Handbook of Toxicologic Pathology (3 edn) (Haschek, W.M. et al. eds).
21. Vissink, A. et al. (1992) Contrasting dose-rate effects of gamma-irradiation on rat salivary gland function. Int J Radiat Biol 61 (2), 275-82.
22. Dreyer, J.O. et al. (1989) [Radiation-induced sialadenitis. Stage classification and immunohistology]. Pathologe 10 (3), 165-70.
23. Marmary, Y. et al. (2016) Radiation-Induced Loss of Salivary Gland Function Is Driven by Cellular Senescence and Prevented by IL6 Modulation. Cancer Res 76 (5), 1170-80.
24. Emmerson, E. et al. (2018) Salivary glands regenerate after radiation injury through SOX2-mediated secretory cell replacement. EMBO Mol Med 10 (3).
25. Zeilstra, L.J. et al. (2000) Radiation induced cell loss in rat submandibular gland and its relation to gland function. Int J Radiat Biol 76 (3), 419-29.
26. Song, E.C. et al. (2018) Genetic and scRNA-seq Analysis Reveals Distinct Cell Populations that Contribute to Salivary Gland Development and Maintenance. Sci Rep 8 (1), 14043.

27. Coppes, R.P. et al. (2002) Comparison of radiosensitivity of rat parotid and submandibular glands after different radiation schedules. Radiother Oncol 63 (3), 321-8.
28. Weng, P.L. et al. (2018) Limited Regeneration of Adult Salivary Glands after Severe Injury Involves Cellular Plasticity. Cell Rep 24 (6), 1464-1470.e3.
29. De Ryck, T. et al. (2015) Dynamic Shifts in the Oral Microbiome during Radiotherapy. Clinical Research in Infectious Diseases 2 (1), 1013.
30. Bradley, P.J. and McGurk, M. (2013) Incidence of salivary gland neoplasms in a defined UK population. Br J Oral Maxillofac Surg 51 (5), 399-403.
31. Sood, S. et al. (2016) Management of Salivary Gland Tumours: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol 130 (S2), S142-s149.
32. Colella, G. et al. (2015) Meta-analysis of surgical approaches to the treatment of parotid pleomorphic adenomas and recurrence rates. J Craniomaxillofac Surg 43 (6), 738-45.
33. Stodulski, D. et al. (2015) Facial nerve grading after parotidectomy. Eur Arch Otorhinolaryngol 272 (9), 2445-50.
34. Zhang, S.E. et al. (2014) Reinnervated nerves contribute to the secretion function and regeneration of denervated submandibular glands in rabbits. Eur J Oral Sci 122 (6), 372-81.
35. Yeh, C.K. et al. (1998) Impact of aging on human salivary gland function: a community-based study. Aging (Milano) 10 (5), 421-8.
36. Yamauchi, Y. et al. (2017) Relationship between hyposalivation and oxidative stress in aging mice. J Clin Biochem Nutr 61 (1), 40-46.
37. Miyagi, Y. et al. (2019) Submandibular gland-specific inflammaging-induced hyposalivation in the male senescence-accelerated mouse prone -1 line (SAM-P1). Biogerontology 20 (4), 421-432.
38. Yamakoshi, K. et al. (2015) Dysregulation of the Bmi-1/p16(Ink(4)a) pathway provokes an aging-associated decline of submandibular gland function. Aging Cell 14 (4), 616-24.
39. Choi, J.S. et al. (2013) Analysis of age-related changes in the functional morphologies of salivary glands in mice. Arch Oral Biol 58 (11), 1635-42.
40. Enoki, N. et al. (2007) Age-dependent changes in cell proliferation and cell death in the periodontal tissue and the submandibular gland in mice: a comparison with other tissues and organs. J Mol Histol 38 (4), 321-32.
41. Guneri, P. et al. (2011) In vitro antimicrobial effects of commercially available mouth-wetting agents. Spec Care Dentist 31 (4), 123-8.
42. Barbe, A.G. et al. (2019) Efficacy of a newly developed mouth gel for xerostomia relief-A randomized double-blind trial. Oral Dis 25 (6), 1519-1529.
43. Baker, J.L. et al. (2017) Ecology of the Oral Microbiome: Beyond Bacteria. Trends Microbiol 25 (5), 362-374.
44. Hou, J. et al. (2018) Distinct shifts in the oral microbiota are associated with the progression and aggravation of mucositis during radiotherapy. Radiother Oncol 129 (1), 44-51.
45. Furness, S. et al. (2011) Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev (12), Cd008934.
46. Lysik, D. et al. (2019) Artificial Saliva: Challenges and Future Perspectives for the Treatment of Xerostomia. Int J Mol Sci 20 (13).
47. Niemirowicz, K. et al. (2017) Formulation and candidacidal activity of magnetic nanoparticles coated with cathelicidin LL-37 and ceragenin CSA-13. Sci Rep 7 (1), 4610.
48. Takesh, T. et al. (2018) Effects of a Novel Formulation on Oral Biofilm, pH Buffering, and Gingival Health in Patients with Dry Mouth. Int J Dent 2018, 2748274.
49. Knox, S.M. et al. (2013) Parasympathetic stimulation improves epithelial organ regeneration. Nat Commun 4, 1494.
50. Takakura, A.C. et al. (2003) Central muscarinic receptors signal pilocarpine-induced salivation. J Dent Res 82 (12), 993-7.
51. Chambers, M.S. et al. (2007) Open-label, long-term safety study of cevimeline in the treatment of postirradiation xerostomia. Int J Radiat Oncol Biol Phys 69 (5), 1369-76.
52. Cifuentes, M. et al. (2018) Pilocarpine and artificial saliva for the treatment of xerostomia and xerophthalmia in Sjogren syndrome: a double-blind randomized controlled trial. Br J Dermatol 179 (5), 1056-1061.
53. Cheng, C.Q. et al. (2016) Efficacy and safety of pilocarpine for radiation-induced xerostomia in patients with head and neck cancer: A systematic review and meta-analysis. J Am Dent Assoc 147 (4), 236-43.
54. Chitapanarux, I. et al. (2008) Effect of oral pilocarpine on post-irradiation xerostomia in head and neck cancer patients: a single-center, single-blind clinical trial. J Med Assoc Thai 91 (9), 1410-5.

55. Hooper, P. et al. (1997) The use of salivary stimulant pastilles to improve compliance in women taking oxybutynin hydrochloride for detrusor instability: a pilot study. Br J Urol 80 (3), 414-6.
56. Mao, A.S. and Mooney, D.J. (2015) Regenerative medicine: Current therapies and future directions. Proc Natl Acad Sci U S A 112 (47), 14452-9.
57. Jensen, D.H. et al. (2014) Mesenchymal stem cell therapy for salivary gland dysfunction and xerostomia: a systematic review of preclinical studies. Oral Surg Oral Med Oral Pathol Oral Radiol 117 (3), 335-342.e1.
58. Lim, J.Y. et al. (2013) Intraglandular transplantation of bone marrow-derived clonal mesenchymal stem cells for amelioration of post-irradiation salivary gland damage. Oral Oncol 49 (2), 136-43.
59. Kojima, T. et al. (2011) Regeneration of radiation damaged salivary glands with adipose-derived stromal cells. Laryngoscope 121 (9), 1864-9.
60. Lin, C.Y. et al. (2011) Cell therapy for salivary gland regeneration. J Dent Res 90 (3), 341-6.
61. Khalili, S. et al. (2012) Mesenchymal stromal cells improve salivary function and reduce lymphocytic infiltrates in mice with Sjogren's-like disease. PLoS One 7 (6), e38615.
62. Xu, J. et al. (2012) Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. Blood 120 (15), 3142-51.
63. Lee, J. et al. (2015) Transdifferentiation of mouse adipose-derived stromal cells into acinar cells of the submandibular gland using a co-culture system. Exp Cell Res 334 (1), 160-72.
64. Park, Y.J. et al. (2014) Identification of regulatory factors for mesenchymal stem cell-derived salivary epithelial cells in a co-culture system. PLoS One 9 (11), e112158.
65. Lim, J.Y. et al. (2013) Systemic transplantation of human adipose tissue-derived mesenchymal stem cells for the regeneration of irradiation-induced salivary gland damage. PLoS One 8 (8), e71167.
66. Xiong, X. et al. (2014) Human adipose tissuederived stem cells alleviate radiationinduced xerostomia. Int J Mol Med 34 (3), 749-55.
67. Gronhoj, C. et al. (2018) Safety and Efficacy of Mesenchymal Stem Cells for Radiation-Induced Xerostomia: A Randomized, Placebo-Controlled Phase 1/2 Trial (MESRIX). Int J Radiat Oncol Biol Phys 101 (3), 581-592.
68. Kim, J.W. et al. (2019) Adipose-derived mesenchymal stem cells regenerate radioiodine-induced salivary gland damage in a murine model. Sci Rep 9 (1), 15752.
69. Gronhoj, C. et al. (2017) First-in-man mesenchymal stem cells for radiation-induced xerostomia (MESRIX): study protocol for a randomized controlled trial. Trials 18 (1), 108.
70. Takashi, I. et al. (2019) Anti-inflammatory and vasculogenic conditioning of peripheral blood mononuclear cells reinforces their therapeutic potential for radiation-injured salivary glands. Stem Cell Res Ther 10 (1), 304.
71. Barkholt, L. et al. (2013) Risk of tumorigenicity in mesenchymal stromal cell-based therapies--bridging scientific observations and regulatory viewpoints. Cytotherapy 15 (7), 753-9.
72. Lombaert, I.M. et al. (2008) Rescue of salivary gland function after stem cell transplantation in irradiated glands. PLoS One 3 (4), e2063.
73. Nanduri, L.S. et al. (2013) Salisphere derived c-Kit cell transplantation restores tissue homeostasis in irradiated salivary gland. Radiother Oncol.
74. Nanduri, L.S. et al. (2011) Regeneration of irradiated salivary glands with stem cell marker expressing cells. Radiother Oncol 99 (3), 367-72.
75. Nanduri, L.S. et al. (2014) Purification and ex vivo expansion of fully functional salivary gland stem cells. Stem Cell Reports 3 (6), 957-64.
76. Shin, H.S. et al. (2018) Stem cell properties of human clonal salivary gland stem cells are enhanced by three-dimensional priming culture in nanofibrous microwells. Stem Cell Res Ther 9 (1), 74.
77. Feng, J. et al. (2009) Isolation and characterization of human salivary gland cells for stem cell transplantation to reduce radiation-induced hyposalivation. Radiother Oncol 92 (3), 466-71.
78. Maimets, M. et al. (2016) Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals. Stem Cell Reports 6 (1), 150-62.
79. Pringle, S. et al. (2011) Isolation of mouse salivary gland stem cells. J Vis Exp (48).
80. Lombaert, I.M. et al. (2008) Keratinocyte growth factor prevents radiation damage to salivary glands by expansion of the stem/progenitor pool. Stem Cells 26 (10), 2595-601.
81. Tatsuishi, Y. et al. (2009) Human salivary gland stem/progenitor cells remain dormant even after irradiation. Int J Mol Med 24 (3), 361-6.
82. Maimets, M. et al. (2015) Similar ex vivo expansion and post-irradiation regenerative potential of juvenile and aged salivary gland stem cells. Radiother Oncol 116 (3), 443-8.
83. Pringle, S. et al. (2016) Human Salivary Gland Stem Cells Functionally Restore Radiation Damaged Salivary Glands. Stem Cells 34 (3), 640-52.

84. Neumann, Y. et al. (2012) Long-term cryopreservation model of rat salivary gland stem cells for future therapy in irradiated head and neck cancer patients. Tissue Eng Part C Methods 18 (9), 710-8.
85. Chibly, A.M. et al. (2014) Label-retaining cells in the adult murine salivary glands possess characteristics of adult progenitor cells. PLoS One 9 (9), e107893.
86. Kwak, M. et al. (2016) Identification of Stem Cells in the Secretory Complex of Salivary Glands. J Dent Res 95 (7), 776-83.
87. Kwak, M. and Ghazizadeh, S. (2015) Analysis of histone H2BGFP retention in mouse submandibular gland reveals actively dividing stem cell populations. Stem Cells Dev 24 (5), 565-74.
88. May, A.J. et al. (2018) Diverse progenitor cells preserve salivary gland ductal architecture after radiation-induced damage. Development 145 (21).
89. Bullard, T. et al. (2008) Ascl3 expression marks a progenitor population of both acinar and ductal cells in mouse salivary glands. Dev Biol 320 (1), 72-8.
90. Aure, M.H. et al. (2015) Salivary gland homeostasis is maintained through acinar cell self-duplication. Dev Cell 33 (2), 231-7.
91. Lu, W.Y. et al. (2015) Hepatic progenitor cells of biliary origin with liver repopulation capacity. Nat Cell Biol 17 (8), 971-983.
92. Raven, A. et al. (2017) Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. Nature 547 (7663), 350-354.
93. Yu, S. et al. (2018) Paneth Cell Multipotency Induced by Notch Activation following Injury. Cell Stem Cell 23 (1), 46-59.e5.
94. Strobel, O. et al. (2007) In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. Gastroenterology 133 (6), 1999-2009.
95. Shubin, A.D. et al. (2020) Stress or injury induces cellular plasticity in salivary gland acinar cells. Cell Tissue Res.
96. Sancho, R. et al. (2014) Loss of Fbw7 reprograms adult pancreatic ductal cells into alpha, delta, and beta cells. Cell Stem Cell 15 (2), 139-53.
97. Athwal, H.K. et al. (2019) Sox10 Regulates Plasticity of Epithelial Progenitors toward Secretory Units of Exocrine Glands. Stem Cell Reports 12 (2), 366-380.
98. Tetteh, P.W. et al. (2016) Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. Cell Stem Cell 18 (2), 203-13.
99. Knox, S.M. et al. (2010) Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis. Science 329 (5999), 1645-7.
100. Emmerson, E. et al. (2017) SOX2 regulates acinar cell development in the salivary gland. Elife 6.
101. Deshmukh, V.A. et al. (2013) A regenerative approach to the treatment of multiple sclerosis. Nature 502 (7471), 327-332.
102. Mattingly, A. et al. (2015) Salivary gland development and disease. Wiley Interdiscip Rev Dev Biol 4 (6), 573-90.
103. Xiao, N. et al. (2014) Neurotrophic factor GDNF promotes survival of salivary stem cells. J Clin Invest 124 (8), 3364-77.
104. Peng, X. et al. (2017) Role of glial-cell-derived neurotrophic factor in salivary gland stem cell response to irradiation. Radiother Oncol 124 (3), 448-454.
105. Clevers, H. et al. (2014) Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346 (6205), 1248012.
106. Haara, O. et al. (2011) Ectodysplasin and Wnt pathways are required for salivary gland branching morphogenesis. Development 138 (13), 2681-91.
107. Hai, B. et al. (2010) Wnt/beta-catenin signaling regulates postnatal development and regeneration of the salivary gland. Stem Cells Dev 19 (11), 1793-801.
108. Hai, B. et al. (2012) Concurrent transient activation of Wnt/beta-catenin pathway prevents radiation damage to salivary glands. Int J Radiat Oncol Biol Phys 83 (1), e109-16.
109. Habib, S.J. et al. (2013) A localized Wnt signal orients asymmetric stem cell division in vitro. Science 339 (6126), 1445-8.
110. Wend, P. et al. (2013) Wnt/beta-catenin signalling induces MLL to create epigenetic changes in salivary gland tumours. Embo j 32 (14), 1977-89.
111. Nam, K. et al. (2019) Synergistic effects of laminin-1 peptides, VEGF and FGF9 on salivary gland regeneration. Acta Biomater 91, 186-194.
112. Badea, A.F. et al. (2013) ARFI quantitative elastography of the submandibular glands. Normal measurements and the diagnosis value of the method in radiation submaxillitis. Med Ultrason 15 (3), 173-9.

113. Kamiya, M. et al. (2015) X-Ray-Induced Damage to the Submandibular Salivary Glands in Mice: An Analysis of Strain-Specific Responses. Biores Open Access 4 (1), 307-18.
114. Peters, S.B. et al. (2014) Biocompatible tissue scaffold compliance promotes salivary gland morphogenesis and differentiation. Tissue Eng Part A 20 (11-12), 1632-42.
115. Sugito, T. et al. (2004) Transplantation of cultured salivary gland cells into an atrophic salivary gland. Cell Transplant 13 (6), 691-9.
116. McCullen, S.D. et al. (2012) Anisotropic fibrous scaffolds for articular cartilage regeneration. Tissue Eng Part A 18 (19-20), 2073-83.
117. Grant, R. et al. (2019) Blended electrospinning with human liver extracellular matrix for engineering new hepatic microenvironments. Sci Rep 9 (1), 6293.
118. Ozdemir, T. et al. (2016) Biomaterials-based strategies for salivary gland tissue regeneration. Biomater Sci 4 (4), 592-604.
119. Szlavik, V. et al. (2008) Matrigel-induced acinar differentiation is followed by apoptosis in HSG cells. J Cell Biochem 103 (1), 284-95.
120. McCall, A.D. et al. (2013) Growth factors polymerized within fibrin hydrogel promote amylase production in parotid cells. Tissue Eng Part A 19 (19-20), 2215-25.
121. Joraku, A. et al. (2007) In-vitro reconstitution of three-dimensional human salivary gland tissue structures. Differentiation 75 (4), 318-24.
122. Gao, Z. et al. (2014) Generation of Bioartificial Salivary Gland Using Whole-Organ Decellularized Bioscaffold. Cells Tissues Organs 200 (3-4), 171-80.
123. Zheng, C. et al. (2011) Prevention of radiation-induced salivary hypofunction following hKGF gene delivery to murine submandibular glands. Clin Cancer Res 17 (9), 2842-51.
124. Okazaki, Y. et al. (2000) Acceleration of rat salivary gland tissue repair by basic fibroblast growth factor. Arch Oral Biol 45 (10), 911-9.
125. Grundmann, O. et al. (2010) Restoration of radiation therapy-induced salivary gland dysfunction in mice by post therapy IGF-1 administration. BMC Cancer 10, 417.
126. Limesand, K.H. et al. (2010) Insulin-like growth factor-1 preserves salivary gland function after fractionated radiation. Int J Radiat Oncol Biol Phys 78 (2), 579-86.
127. Redman, R.S. et al. (2009) Dispersed donor salivary gland cells are widely distributed in the recipient gland when infused up the ductal tree. Biotech Histochem 84 (6), 253-60.
128. Delporte, C. et al. (1997) Increased fluid secretion after adenoviral-mediated transfer of the aquaporin-1 cDNA to irradiated rat salivary glands. Proc Natl Acad Sci U S A 94 (7), 3268-73.
129. Shan, Z. et al. (2005) Increased fluid secretion after adenoviral-mediated transfer of the human aquaporin-1 cDNA to irradiated miniature pig parotid glands. Mol Ther 11 (3), 444-51.
130. O'Connell, A.C. et al. (1999) Safety and efficacy of adenovirus-mediated transfer of the human aquaporin-1 cDNA to irradiated parotid glands of non-human primates. Cancer Gene Ther 6 (6), 505-13.
131. Lai, Z. et al. (2016) Aquaporin gene therapy corrects Sjogren's syndrome phenotype in mice. Proc Natl Acad Sci U S A 113 (20), 5694-9.
132. Arany, S. et al. (2013) Nanoparticle-mediated gene silencing confers radioprotection to salivary glands in vivo. Mol Ther 21 (6), 1182-94.
133. Varghese, J.J. et al. (2018) Retroductal Nanoparticle Injection to the Murine Submandibular Gland. LID - 10.3791/57521 [doi]. J Vis Exp 3 (1940-087X (Electronic)), 135.
134. Medina, V.A. et al. (2011) Histamine prevents functional and morphological alterations of submandibular glands induced by ionising radiation. Int J Radiat Biol 87 (3), 284-92.
135. Baum, B.J. et al. (2012) Early responses to adenoviral-mediated transfer of the aquaporin-1 cDNA for radiation-induced salivary hypofunction. Proc Natl Acad Sci U S A 109 (47), 19403-7.
136. Alevizos, I. et al. (2017) Late responses to adenoviral-mediated transfer of the aquaporin-1 gene for radiation-induced salivary hypofunction. Gene Ther 24 (3), 176-186.
137. Corden, A. et al. (2017) Neutralizing antibodies against adeno-associated viruses in Sjogren's patients: implications for gene therapy. Gene Ther 24 (4), 241-244.
138. Vosters, J.L. et al. (2012) TACI-Fc gene therapy improves autoimmune sialadenitis but not salivary gland function in non-obese diabetic mice. Oral Dis 18 (4), 365-74.
139. Lewis-McDougall, F.C. et al. (2019) Aged-senescent cells contribute to impaired heart regeneration. Aging Cell 18 (3), e12931.
140. Sadtler, K. et al. (2016) Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science 352 (6283), 366-70.
141. Kawakami, M. et al. (2013) Functional transplantation of salivary gland cells differentiated from mouse early ES cells in vitro. Hum Cell 26 (2), 80-90.

142. Tanaka, J. et al. (2018) Generation of orthotopically functional salivary gland from embryonic stem cells. Nat Commun 9 (1), 4216.
143. Ono, H. et al. (2015) Regenerating Salivary Glands in the Microenvironment of Induced Pluripotent Stem Cells. Biomed Res Int 2015, 293570.
144. Maruyama, C.L. et al. (2019) Comparing human and mouse salivary glands: A practice guide for salivary researchers. Oral Dis 25 (2), 403-415.
145. Tarlow, B.D. et al. (2014) Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell 15 (5), 605-18.

Highlights

- The vast majority of the 550,000 patients who undergo head and neck cancer radiotherapy annually and more than 4 million Sjögren's Syndrome patients worldwide live with salivary dysfunction
- There currently exists no FDA-approved permanent solution to salivary gland hypofunction and resulting xerostomia
- Stem cell transplantation to replace functional salivary 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

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 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, 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 non-invasive and abundant source of stem cells**.** However, the current challenges in iPSC use include cells retaining some epigenetic memory of the tissue they were derived from, 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.

Figure 1

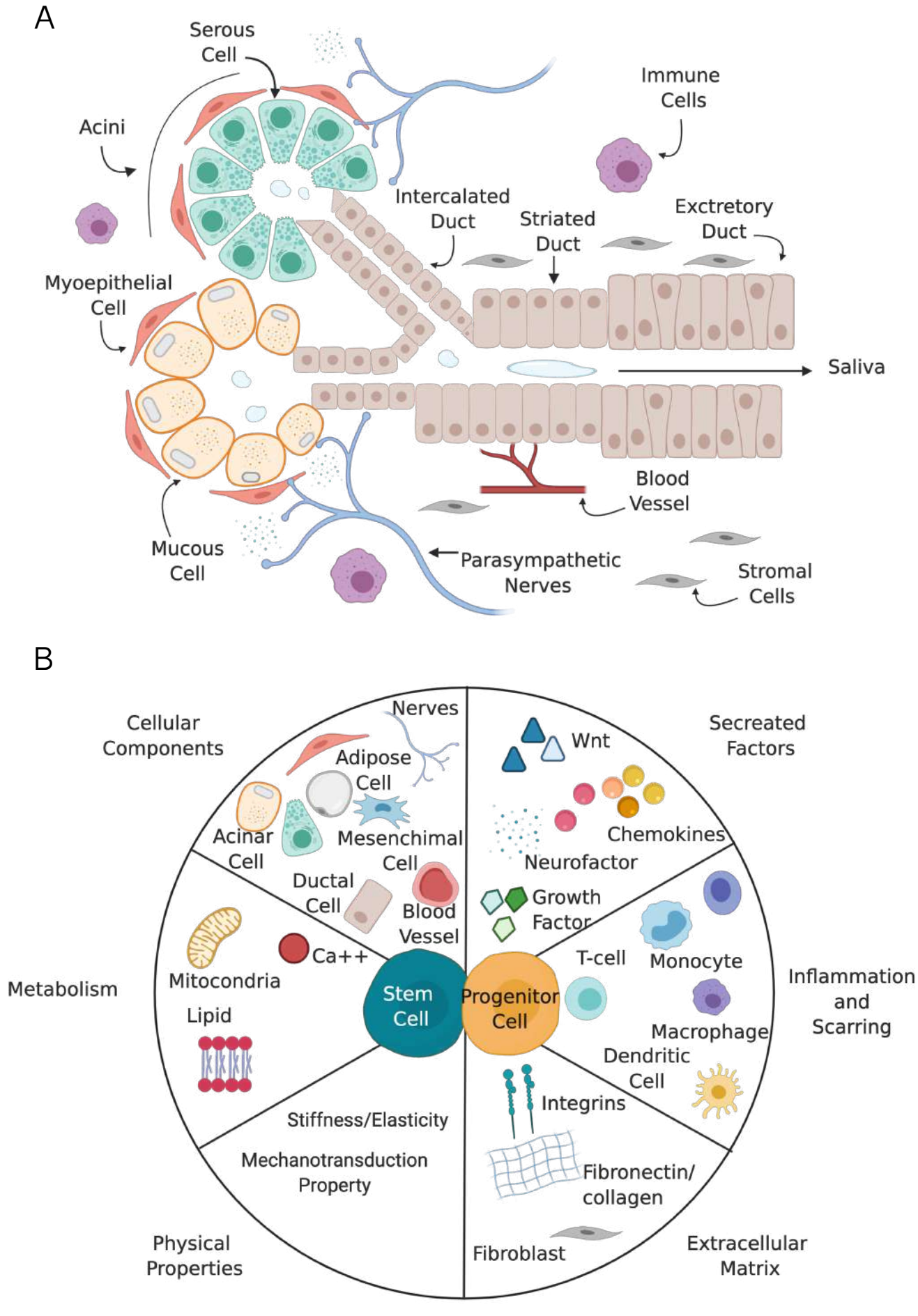

A
Serous Cell
Acini
Immune Cells
Intercalated Duct
Striated Duct
Exctretory Duct
Myoepithelial Cell
Saliva
Blood Vessel
Mucous Cell
Parasympathetic Nerves
Stromal Cells
B
Cellular Components
Nerves
Adipose Cell
Acinar Cell
Mesenchimal Cell
Ductal Cell
Blood Vessel
Secreated Factors
Wnt
Chemokines
Neurofactor
Growth Factor
Metabolism
Mitocondria
Ca++
Lipid
Stem Cell
Progenitor Cell
T-cell
Monocyte
Macrophage
Dendritic Cell
Inflammation and Scarring
Stiffness/Elasticity
Mechanotransduction Property
Integrins
Fibronectin/ collagen
Physical Properties
Fibroblast
Extracellular Matrix

Figure 2

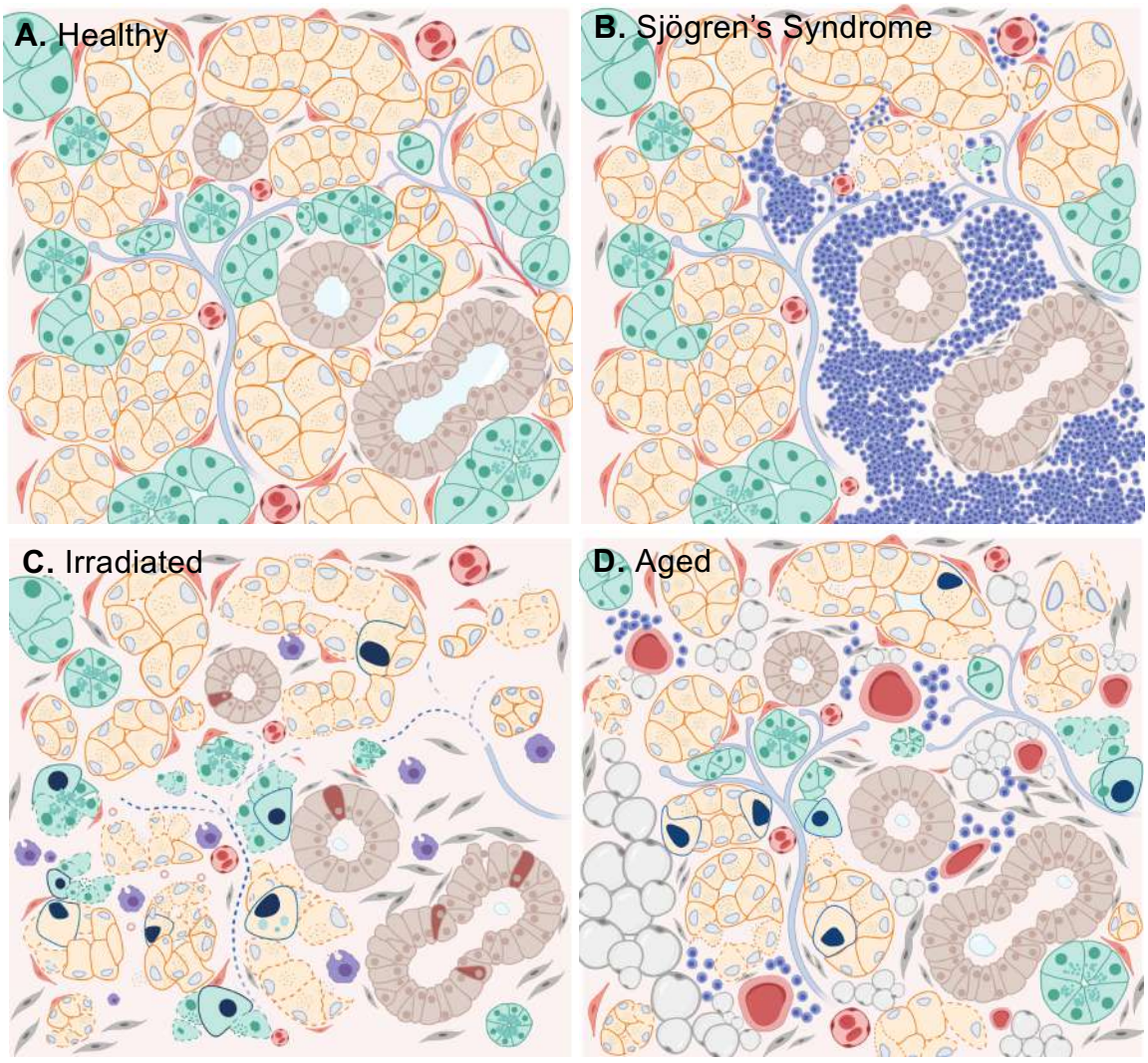

A. Healthy
B. Sjögren's Syndrome
C. Irradiated
D. Aged
Key
Mucous Acinar Cell
Duct Stem/Progenitor Cell
Adipose Cell
Parasympathetic Nerves
Serous Acinar Cell
Duct Cell
Fibroblast
Macrophage
Myoepithelial Cell
Senescent Cell
Neutrophil
Enlarged Blood Vessel
Damaged Mucous Acinar Cell
Damaged Serous Acinar Cell

Figure 3

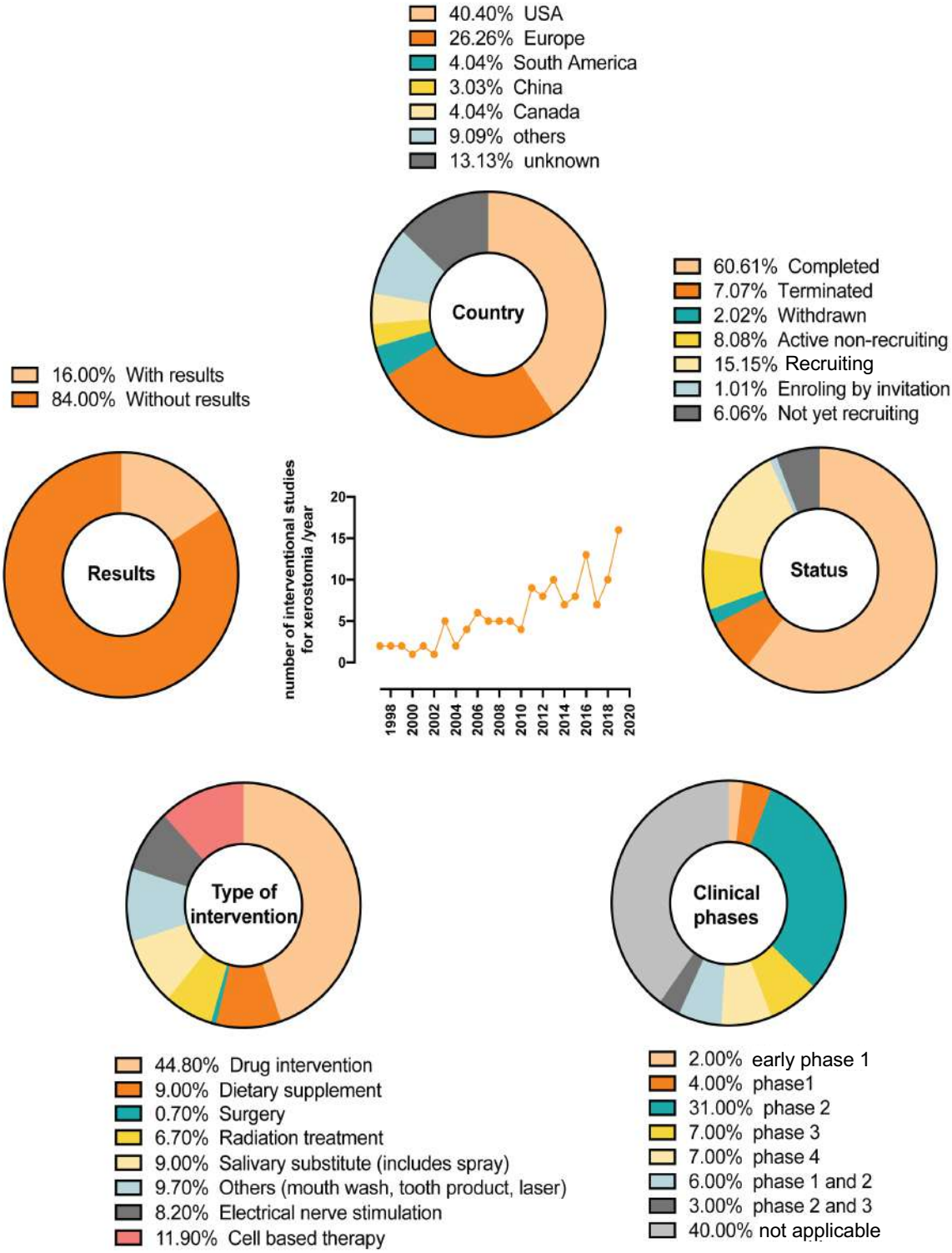
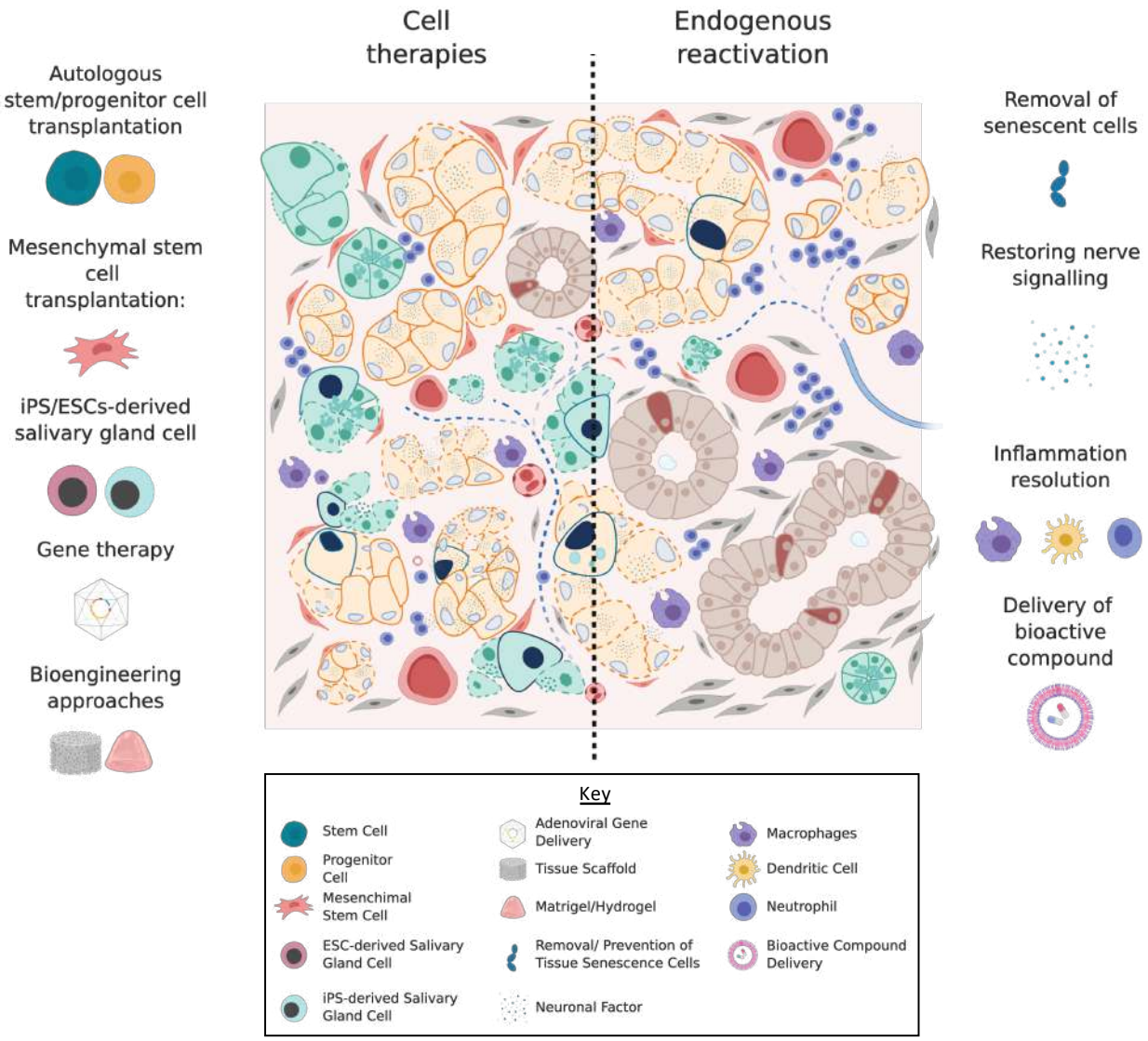

40.40% USA
26.26% Europe
4.04% South America
3.03% China
4.04% Canada
9.09% others
13.13% unknown
Country
60.61% Completed
7.07% Terminated
2.02% Withdrawn
8.08% Active non-recruiting
15.15% Recruiting
1.01% Enroling by invitation
6.06% Not yet recruiting
16.00% With results
84.00% Without results
Results
number of interventional studies for xerostomia /year
20
15
10
5
0
1998
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
2020
Status
Type of intervention
Clinical phases
44.80% Drug intervention
9.00% Dietary supplement
0.70% Surgery
6.70% Radiation treatment
9.00% Salivary substitute (includes spray)
9.70% Others (mouth wash, tooth product, laser)
8.20% Electrical nerve stimulation
11.90% Cell based therapy
2.00% early phase 1
4.00% phase1
31.00% phase 2
7.00% phase 3
7.00% phase 4
6.00% phase 1 and 2
3.00% phase 2 and 3
40.00% not applicable

Figure 4


Cell therapies
Endogenous reactivation
Autologous stem/progenitor cell transplantation
Mesenchymal stem cell transplantation:
iPS/ESCs-derived salivary gland cell
Gene therapy
Bioengineering approaches
Removal of senescent cells
Restoring nerve signalling
Inflammation resolution
Delivery of bioactive compound
Key
Stem Cell
Progenitor Cell
Mesenchimal Stem Cell
ESC-derived Salivary Gland Cell
iPS-derived Salivary Gland Cell
Adenoviral Gene Delivery
Tissue Scaffold
Matrigel/Hydrogel
Removal/ Prevention of Tissue Senescence Cells
Neuronal Factor
Macrophages
Dendritic Cell
Neutrophil
Bioactive Compound Delivery