Chapter

Salivary Gland Radio-Protection, Regeneration and Repair: Innovative Strategies

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Abstract

Saliva has a critical role in the maintenance of oral, dental and general health and well-being. Alteration(s) in the amount/quantity and/or quality of secreted saliva may induce the development of several oro-dental variations, thereby negatively-impacting overall quality of life. Diverse factors may affect the process of saliva production and quantity/quality of secretion, including medications, systemic or local pathologies and/or reversible/irreversible damage. Indeed, chemo- and/or radio-therapy, particularly, in cases of head and neck cancer, for example, are well-documented to induce serious damage and dysfunction to the *radio-sensitive* salivary gland tissue, resulting in hypo-salivation, xerostomia (dry mouth) as well as numerous other adverse intra–/extra-oral, medical and quality-of-life issues. Although a single governing mechanism of radiation-induced salivary gland tissue damage and dysfunction has not been yet elucidated, the potential for a synergy in radio-protection (mainly, and possible -reparation) via a combinatorial approach of mechanistically distinct strategies, has been suggested and explored over the years. This is, undoubtfully, in parallel to the ongoing efforts in improving the precision, safety and efficacy of radiotherapy protocols/outcomes, as well as in developing new technological and pharmaceutical alternatives, topics covered in this chapter.

Keywords: radioprotection, salivary gland, xerostomia, head and neck cancer, oro-dental health

1. Introduction

It is well recognized that the incidence of cancer, the second leading cause of death, globally, is increasing, an ongoing major burden of disease and public health burden, World-wide. While there were 14.1 million cancer cases reported in 2012, the World Health Organization (WHO) estimated about 1 in 6 deaths is due to cancer, with 9.6 million such deaths reported in 2018. In the United States, today, cancer is the second leading, after heart disease, cause of death amongst men and women, with over 1 million new cases diagnosed, annually [1].

Despite a reduction in tobacco consumption and the significant modern advancements in medicine, the number of new cancer cases, per year, is projected to rise to 22.2 million by 2030 [1]. Cancers, often squamous cell carcinomas/neoplasms, that involve the oral cavity, nostrils, paranasal sinuses, naso–/oro–/hypo-pharynx,

larynx, and the salivary glands, are commonly/collectively (despite their heterogeneity) termed head and neck cancers (HNC), which, together are responsible for nearly 200,000 deaths, a year, World-wide [2]. In the United States alone, HNC represent 4–5% of all cancers, and in Europe, HNC are the sixth most common group of cancers [3].

Besides the alarming incidence and mortality rates, HNC suffer a relatively poor prognosis, overall, whether due to delays in diagnosis, staging, treatment, particulars of the tumor site, onset, type of symptoms and/or efficacy of therapies, to mention a few. Such factors further contribute to permitting the progress and upstaging of the malignant tumor(s) which eventually result in enfeebled survival, despite the application of novel or advanced intensive therapeutic regimens. Briefly, treatment, often a multi-disciplinary case-specific approach, can employ chemo−/radio−/immune-therapy, surgery, or combinatorial strategies [4].

Herein, radiotherapy (RT), whether radical or prophylactic, remains a mainstay of HNC treatment, especially in light of modern improvements in precisely targeting and delivering the required radiation doses to the tumor, thereby allowing additional sparing of normal/healthy surrounding tissue(s), greatly reducing side or adverse effects of radiation, and consequently improving the quality of life (QoL) of patients as well as their families [5–8]. IMRT (intensity-modulated radiotherapy), VMAT (volumetric modulated arc therapy) and particle (ion-based) therapy are perhaps fine examples of modern high-precision RT [7].

RT, in general, aims to realize *localized* destruction and control of the target tumor (−cells) and halt of the reproductive potential, while minimizing toxicity onset. Specifically, high-energy radiation is deposited, causing DNA strands to break thereby damaging the cell genome either directly or indirectly (via free-radical production) and subsequently resulting in apoptosis, mitotic cell death, and tissue hypoxia, through different cascades and processes [5, 7]. Depending on the radiation dose and tissue turnover, amongst other factors, RT can almost always be expected to result in a range of side effects, of which some are reversible and others are irreversible (**Figure 1**). Indeed, HNC and oral squamous cell carcinoma (OSCC) patients receiving RT often experience pain, taste disturbances, difficulties in mastication and deglutition (swallowing) and suffer from mucositis, fungal infections, dental decay, alterations in speech, all of which are mainly due to or linked to salivary gland dysfunction which in turn results in hyposalivation and xerostomia [9–12].

Herein, xerostomia, a dry mouth sensation, is one of the main complications and complaints for HNC patients receiving RT, mainly as a sequela of the un-avoidable damage to the parotid and sub-mandibular glands (both produce over 80% of saliva) anatomically located with the radiation zone [8, 12]. Inflammation, fibrosis, atrophy and the reduced wound healing response, *i.e.* reparative and regenerative capacity of the glands, mainly due to lack of *functional* salivary gland stem/progenitor cells post-irradiation, render the *inevitable* radiotherapy-induced salivary gland damage and dysfunction, whether occurring early or late, a significant impediment to the QoL and survival of HNC and OSCC patients [10, 13, 14].

Therefore, besides modern advancements in radiation engineering technologies, ample pharmacological and pharmaceutical solutions have been explored [14]. Accumulating knowledge in understanding underlying signaling pathways, cellular and tissue responses, spatio-temporally, fuel the continuing efforts aimed to explore, develop and translate novel solutions to support in the prevention (and treatment of) radiation-induced side-effects and damage of salivary glands, a main focus of this chapter, designed to provide the *clinical* reader with a summary of relevant literature and recent innovative developments in salivary gland radioprotection and potential salivary gland repair, post-RT.

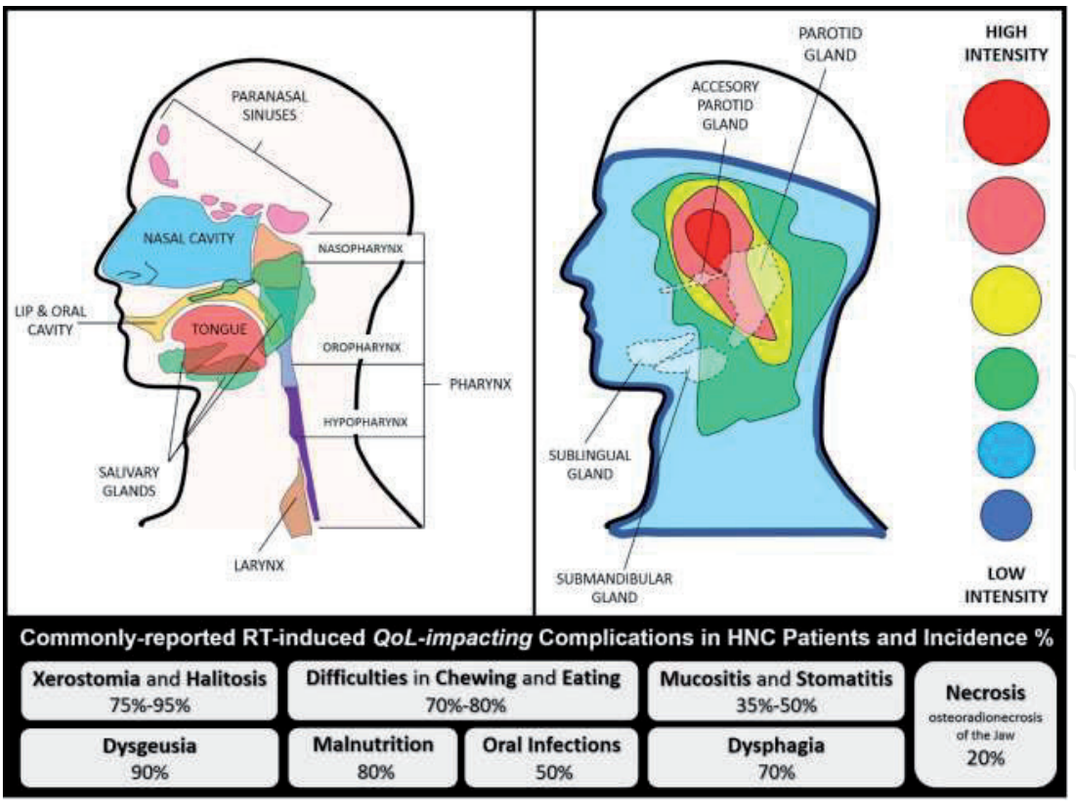


Figure 1.
Head and neck cancers regions and irradiation intensity risk during HNC radiotherapy.

2. Saliva and salivary glands: pre-, during- and post-RT

Briefly, exocrine salivary glands are classified as either major (parotid, sub-mandibular and sub-lingual) or minor (labial and buccal gland, glosso-palatine gland, and palatine and lingual) glands. Anatomically, all three major glands are highly vascularized, innervated and are architecturally similar featuring a ductal structure with a secretory/excretory (saliva-producing acini surrounded by myo-epithelial cells, myo-fibroblasts, immune cells, stromal cells, endothelial cells and nerve fibers) opening into the oral cavity/mouth [15]. The glands differ in their type of acinar cells and as a result, in the type of produced saliva. While the parotid is composed of only serous acini thereby producing watery saliva, the sub-mandibular and sub-lingual glands contain a mix of serous and mucous (glycoprotein- rich) acini, thereby producing saliva of a different composition, a seromucous secretion. Secretion of saliva is stimulated by the sympathetic (proteins) and parasympathetic (serous/ions) branches of the autonomic nervous system [15, 16].

Saliva is basically an oral lubricant fluid with multiple digestive functions critical for oro-dental health, QoL and general well-being. It is composed of a complex mixture of water (99%), electrolytes (sodium, potassium, calcium, magnesium, etc. ...), mucins, proteins, white blood cells, epithelial cells, immunoglobulins, anti-microbials/−bacterials and enzymes (1%) [16–18]. Hence, saliva is essential for moistening, chewing, swallowing and chemically-digesting foods. It also facilitates speaking, aids the tongue in taste sensing, helps protect the oral mucosa (localized immunity/mucosal resistance) and plays a role in tissue re-mineralization. A healthy adult produces/secretes a daily average of 0.5–1.5 L, at differential rates over the day, and at a near neutral (buffer) pH of 6.7 [15–17, 19–22].

Therefore, alterations in quantity (↓: hypo- or ↑: hyper-salivation) or quality of the secreted/produced saliva are associated to a variety of conditions and diseases

and have been associated with some medications and therapies [23]. For instance, sialorrhea is a general term used for hyper-salivation (or drooling), often as a result of medication, systemic diseases, psychiatric disorders and/or oral pathologies, amongst others [14]. It is also often linked to conditions such as Parkinson's, epilepsy, amyotrophic lateral sclerosis or ALS, cerebral palsy, developmental disabilities, pregnancy and/or drugs including clozapine [16, 24] Common treatments for sialorrhea include surgical intervention, radiation of the salivary glands (to halt and diminish its function) and the use of oral anti-cholinergic drugs (to inhibit saliva production), however with known side or adverse effects. In recent years, numerous studies investigated the use of neuro-toxins, mainly botulinum neurotoxins or BoNTs, which basically are bacterial exotoxins that interfere and block the exocytotic release of vesicular neuro-transmitters cholinergic neuromuscular activity in the target tissue, including commercially-available RimabotulinumtoxinB (RimaBoNT-B, FDA approval in 2000) and IncobotulinumtoxinA (IncoBoNT-A, FDA approval in 2010) in patients suffering sialorrhea, with attractively promising results [24, 25].

On the other hand, salivary gland hypofunction (*progressive* loss of gland function) is commonly described or associated with the reduction of salivary flow and production, quantitatively. Frydrych [26], discussed salivary gland hypofunction etiology and classified causes into seven major areas, developmental, autoimmune/chronic inflammatory, endocrine, neurological/psychiatric, metabolic, infectious and iatrogenic [26]. In a healthy individual, un-stimulated "whole" salivary flow rate is averaged at 0.35 mL saliva per minute, with abnormalities indicated if the rate drops. For example, one of the most prevalent and studied diseases or disorders of the salivary gland is Sjögren's syndrome (SS), a chronic auto-immune inflammatory reaction characterized by lymphocytic infiltration of the exocrine glands (mostly to the salivary or lacrimal glands), which generates a significant reduction in salivary flow rate - to below 0.1 mL whole saliva per minute secreted, un-stimulated [27]. It is perhaps noteworthy herein that *whole* saliva indicates the collection of saliva (secreted from all salivary glands) present in the mouth. Other quantification techniques require direct collection from the specific gland. Moreover, often is reported in diagnosing SS that only un-stimulated whole saliva flow rates are used.

Hypo-salivation, therefore, is salivary flow rate reduction, quantified, clinically via sialometry. Xerostomia, on the other hand, is the reported perception or sensation, subjectively, of oral dryness. Hypo-salivation may or may not be accompanied by xerostomia, and vice versa. Dryness in the mouth can be a side-effect of medications or due to diseases such as HIV/AIDS, diabetes, hypertension and/or other factors including smoking, dehydration, mouth breathing, aging and/or head and neck irradiation [14, 16, 23, 28, 29]. Indeed, xerostomia is one of the most commonly reported (and expected) complications of RT (during and after RT) for HNC, and as mentioned earlier, mainly as a predictable consequence to the significant damage (and generated inflammatory immune response) caused to the salivary glands which are located and included within the RT-zone or field [30–32].

RT, besides impairing salivary gland function and salivary flow rate, impacts the quality of the secreted saliva, given the loss or atrophy of acinar and ductal cells and granules (and stem/stromal and progenitor cells) and the consequential morphological changes to salivary fluid quality (including pH and buffering capacity), thereby affecting the essential protective, functional and overall physiologic processes (**Figure 2**). Such damage [32] and impact can appear as soon as one week after the first radiation therapy session (*acute RT-induced damage is due to a disturbance in the involved signal transduction pathways on the cell membrane*). Progressive

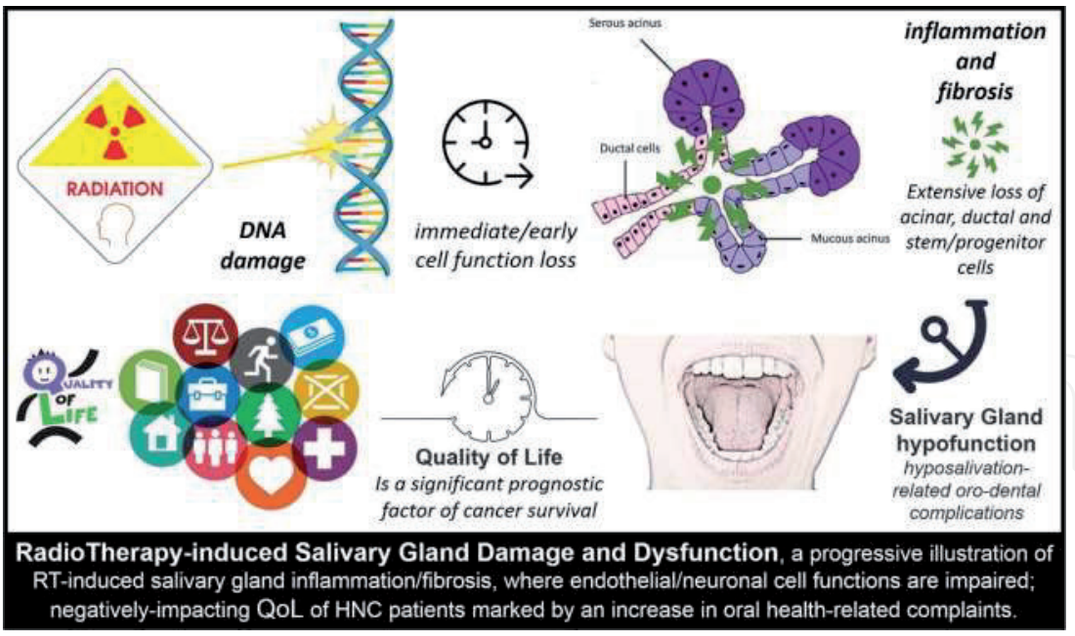


Figure 2.
Progression of RT-induced salivary gland damage and dysfunction in HNC patients.

decrease in salivary gland function is evident with more RT sessions (*delayed or late RT-induced damage is due to apoptosis-driven parenchymal cell loss, inflammation, blood vessel dilation and function loss, nerve injury and reduced parasympathetic nervous function, and fibrosis)* rendering rescue, repair and regeneration rather challenging [33–35].

As a result, the QoL of a large proportion of patients receiving RT is severely compromised [36, 37], with thicker or more viscous saliva and xerostomia leading in reported complaints [38]. Indeed, RT-related biochemical and proteomic alterations where several key glycoproteins, proteins and other molecules are affected have been identified [31, 39]. For example, Jehmlich *et al.* [40], discussed such variations post-RT, detected significant alterations in 48 proteins and highlighted the development of oral mucositis as a result of salivary gland dysfunction. Psychosocial and emotional impact on QoL of HNC patients, especially the elderly [41], where they experience and suffer from a compromised ability to and taste, chew, and swallow foods extended to their forced switching of dietary preferences to soft and carbohydrate-rich foods, thereby resulting in serious nutritional deficiencies [38, 40, 41]. Hyposalivation and sequential xerostomia also affect speaking and communication abilities, and patients experience nocturnal oral discomfort, hence, causing additional stress leading to withdrawal from everyday or day-to-day societal and emotional interactions [42–44].

Furthermore, with the prolonged oral clearance of sugars, the oral mucosa becomes painfully-dry, sticky and more susceptible to infection, the progression of dental caries (tooth decay), gingival and periodontal disease and trauma, accentuating the importance of oro-dental hygiene and care, especially in the elderly patients [41]. Other sequelae include erosion and ulceration of mucosal tissues, oral candidiasis, dysgeusia and dysphagia. Therefore, it is common for HNC patients to suffer from depression, feelings of anguish and anxiety after receipt of the RT protocol [14, 37, 45–49]. While the recovery of irradiated salivary glands at the cellular and molecular has been thus far shown to be limited, salivary recovery post-RT, from our clinical exposure and expertise, is possible, yet a lengthy (> 3 years), dire and capricious process, with underlying mechanisms not yet fully understood.

3. Radiation-induced damage prevention and potential regeneration of salivary glands

Understanding the underlying mechanisms governing cellular and molecular control of salivary gland function is highly pertinent, during- and post-RT, to aid in developing suitable and effective therapies, whether preventive or reparative. To date, it is safe to state that available therapies continue to be symptomatic and no definitive solution or approach has been shown to compensate and/or recover the impairment of salivary glands and function. Life-style modifications, synthetic saliva and/or use of salivary stimulants and sialagogues, suffer shortcomings and are not satisfactory to our patients, as they either only provide temporary (short-term) relief or might have other disquieting side-effects. Hence, global attention has been diverted to seek and develop alternative novel methods, tools and therapies, to offer to HNC patients undergoing RT, that can provide superior long-term efficacy. Herein, tissue engineering, regenerative medicine, pharmaceutics and nanotechnology may contribute.

4. Tissue engineering and reparative/regenerative medicine: current regimens and strategies

Several tissues and organs are highly sensitive to irradiation, such as the skin, esophagus and bone marrow. However, the salivary glands are intricately radiosensitive, given their highly-differentiated cell content marked with a very low or slow proliferative rate [50]. This can help explain why the salivary glands, in specific, are somewhat unique in their early- and delayed-effects post-RT, when compared to other tissues and organs. Nonetheless, salivary gland dysfunction and/or hypofunction has been shown, in some cases, to be reversible. Such treatment intervention is multi-factorial and highly-dependent on original causality, for example, in cases of alcohol abuse and dehydration or hypothyroidism. RT-induced salivary gland damage and dysfunction is a far more challenging scenario. Auto-immune/chronic inflammatory diseases, such as SS or systemic lupus erythematosus also result in irreversible damage to the salivary glands [26].

Today, as mentioned earlier, only palliative and efficacy-limited regimens are commercially-available [47]. **Tables 1–4** highlight a selection of various radio-protection strategies, at different stages of development, pre-clinically (*in vitro* and in *in vivo* testing) and clinical (human clinical trials). Briefly, database search was performed in PubMed-indexed articles using a multi-search of the following key-words: "Salivary Glands AND Radioprotection [Title/Abstract]", "Salivary Glands AND Radioprotection [MeSH]", "Salivary AND Glands AND Radioprotection [Title/Abstract]", "Salivary Gland AND Radioprotection [Title/Abstract]", "Salivary AND Gland AND Radioprotection [ALL FIELDS]", "Salivary Glands AND Radioprotection [ALL FIELDS] and "Salivary Gland AND Radioprotection [ALL FIELDS]". Eligibility and inclusion criteria included English articles reporting radio-protection data from *in vitro*, *in vivo* and/or clinical setting/trials. Articles dated back to 1978 up to the search end-date of December 31st of 2019 were analyzed. Reviews, communications or articles with preliminary results were not included in our analysis (**Figure 3**). Herein, our purpose is to screen the available literature and assess the level of development of new strategies, regimens and/or innovative solutions, to provide a usable prior-Art formatted report. Hence, not all included articles, which are tabulated for the reader, were aimed to be presented and dissected to be discussed in detail. This review attempts to provide an overview of the current understanding, status and prospect of salivary gland radioprotection

Agent	Main Findings	Ref
bFGF-PLGA microspheres	Administration of basic Fibroblast Growth Factor (bFGF) prior to and immediately after irradiation, partially protected (44%) the rat parotid gland.	[51]
pH-responsive nanoparticles for active siRNAs delivery	Introduction of siRNAs specifically targeting the Pkcδ or Bax genes significantly blocked the induction of these pro-apoptotic proteins that normally occurs post-irradiation in cultured salivary gland cells. Level of cell death from subsequent irradiation was significantly decreased.	[52]
rhHGF	Treatment of irradiated hPTS with recombinant human Hepatocyte Growth Factor (rhHGF) restored salivary marker expression and secretory function of hPTS. Changes in the phosphorylation levels of apoptosis-related proteins through HGF-MET axis inhibited irradiation-induced apoptosis.	[53]
TIGAR over-expression	*TIGAR* (a p53-inducible regulator of glycolysis and apoptosis) over-expression could diminish the radio-sensitivity of Hs 917.T cells, and decrease the autophagy level induced by ionizing irradiation.	[54]

Table 1.
Radioprotection of salivary glands, in vitro.

MICE		
Agent	**Main Findings**	**Ref**
Keratinocyte Growth Factor-1 (KGF-1)	Local delivery of keratinocyte growth factor-1 into irradiated salivary glands protected RT-induced salivary cell damage, suppressed p53-mediated apoptosis and prevented salivary hypofunction.	[55]
pH-responsive nanoparticles complexed with siRNAs	Knockdown of Pkcδ reduced the number of apoptotic cells during the acute phase of irradiation damage and also markedly improved salivary secretion at 3 months.	[52]
Dasatinib / Imatinib	Delivery of dasatinib or imatinib resulted in >75% protection/rescue of salivary gland function at 60 days end-point. Continuous dosing with dasatinib extended protection to at least 5 months and was correlated with histologic evidence of regenerated salivary gland acinar cells.	[56]
Human Adipose tissue-derived Mesenchymal Stem Cells (AdMSCs)	Local transplantation of AdMSCs improved tissue remodeling following irradiation-induced damage in salivary gland tissue. The use of a carrier enhanced the effects of AdMSC-mediated cellular protection against irradiation via paracrine secretion.	[57]
Botulinum Toxins (BTX)	Irradiated mice showed a 50% reduction in salivary flow after 3 days, whereas mice pre-injected with BTX had 25% reduction in salivary flow rate ($p < 0.05$). BTX pre-treatment ameliorates RT-induced salivary gland dysfunction.	[58]
AdMSCs secretome	Secretome modulated by hypoxic conditions to contain therapeutic factors contributed to salivary gland tissue re-modeling and demonstrated a potential to improve consequences of RT-induced salivary hypofunction.	[59]
Resveratrol (RES)	Administration of RES reversed the reduction of saliva secretion induced by irradiation and restored salivary amylase and superoxide dismutase activity. RES can protect salivary glands against the negative effects of irradiation.	[60]

MICE		
Agent	**Main Findings**	**Ref**
Amifostine	Amifostine alleviated the effects of irradiation on the bio-functions of cells, such as organelles, highly-involved in the secretory process. Amifostine can alleviate xerostomia caused by the late or delayed effects of irradiation.	[61]
Serotype 5 Adenoviral (Ad5) vector-mediated transfer of basic Fibroblast Growth Factor (AdbFGF) or Vascular Endothelial Growth Factor (AdVEGF) complementary DNAs	Single local administration of a modest dose (5×10^9 particles/gland) of a serotype 5 adenovirus (Ad5) vector encoding either bFGF or VEGF prior to irradiartion, prevents rapid micro-vessel density loss in salivary glands and reduces the loss in salivary flow rate (as measured 8 weeks post-RT).	[62]
Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl)	Tempol treatment was found to protect salivary glands significantly against radiation damage (approximately 60% improvement), with no tumor protection observed.	[63]
Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl)	Tempol treatment pre-irradiation significantly reduced RT-induced salivary hypofunction (approximately 50–60%). Tempol (I.V. or S.C.) administration also showed significant radio-protection. Topical use of tempol, either as a mouthwash or gel, was also reported to be radioprotective.	[64]
Isoproterenol (IPR)	IPR, stimulates adenylate cyclase/cyclic AMP (AC/cAMP) to increase the level of cAMP,25 and then increases cellular membrane ion permeability, ion active transport, and protein bio-synthesis. These events, together with the release of heavy metals, appear to reduce irradiation injury.	[65]
Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl)	Irradiation resulted in a dose-dependent reduction of salivary flow rate in this mouse model.	[66]
WR-2721 WR-3689 WR779 13	Tumors examined take up less WR-3689 than the other two protectors. In RIF-1 tumor, WR-3689 is taken up most avidly, but the three drugs tend to be equally protective.	[67]
WR-2721	There is potential for protecting dose-limiting, late-responding normal tissue in the RT of human tumors with both neutrons and conventional radiotherapy.	[68]
WR-1065	Localized delivery to salivary glands markedly improved radioprotection at the cellular level. Also, mitigated the adverse side-effects associated with systemic administration.	[69]
Hypoxia pre-conditioned human Adipose tissue-derived Mesenchymal Stem Cells (hAdMSCs-HPX)	Results suggest that hAdMSCs-HPX protect salivary glands from RT-induced apoptosis, and preserve acinar structure and functions via the activation of FGFR-PI3K signaling by actions of hAdMSC-secreted factors, including FGF-10.	[70]
Entolimod	At days 8 and 15, entolimod treatment led to noticeable mitigation of damage in salivary gland tissue. Treatment 1 hr. post-RT irradiation seems more effective than 30 min pre-RT.	[71]

MICE		
Agent	**Main Findings**	**Ref**
Statins (Simvastatin)	Administration of Simvastatin could delay and reduce the extent of elevation/over-expression of TGF-β1, which in turn protects the submandibular glands from RT-induced injury.	[72]
RAT		
Agent	**Main Findings**	**Ref**
Se, Zn and Mn + *Lachesis muta* venom (O-LM)	O-LM prevented permanent submandibular gland alterations demonstrating promising results in radioprotection and recovery from RT-induced injury.	[73]
Pilocarpine, Methacholine, Reserpine and Methacholine + Reserpine	Pre-treatment with pilocarpine or methacholine improved all measured glandular functions. Pre-treatment with a combination of reserpine and methacholine showed additive protective effects on submandibular gland function, signifying cooperation of muscarinic and alpha-adrenergic receptors.	[74]
Phenylephrine Isoproterenol Methacholine or Methacholine + Phenylephrine	Pre-treatment with phenylephrine, isoproterenol and methacholine combined with phenylephrine resulted in less irradiation damage to parotid gland functions as indicated by quantified lag phase and flow rate.	[75]
WR-2721	WR-2721 provided a significant degree of protection for all glandular functional parameters including gland weight.	[76]
cAMP	The demonstrated substantial protective effect of exogenously-administered cAMP on the parotid gland supports the previously-suggested radioprotection mechanism by the beta-adrenergic agonist isoproterenol, which is known to elevate endogenous intracellular cAMP.	[77]
WR-2721 Isoproterenol	The aminothiol WR-2721 and beta-adrenergic agonist isoproterenol both conferred considerable radioprotection to the rat parotid gland. Isoproterenol acts on the beta-receptor, and its specific antagonist, propranolol, eliminated the protective effect of isoproterenol, thereby implicating the beta-receptor and cAMP in the radioprotection mechanism.	[78]
WR-2721	While non-protected glands suffered a drastic reduction in the amount of acinar tissue, ducts and blood vessels exhibited only minor morphological changes. Herein, WR-2721 protected the glands with similar signs of damage yet to a much lesser degree, in comparison.	[79]
WR-2721	WR-2721 protected against the acute phase of irradiation damage manifested during the first week post-RT. The drug also protected against chronic damage, appearing later.	[80]
Thymol	Thymol at a dose of 50 mg/Kg significantly impacted (positively) salivary gland dysfunction caused by ionizing irradiation. Short- and late- side effects of RT on the salivary glands were considered reduced by Thymol in those rats.	[81]

RAT		
Agent	**Main Findings**	**Ref**
TLK1B	After a single fraction of 16 Gy, the decline in salivary function at 8 weeks was less pronounced in TLK1B-treated animals (40%) when compared to saline-treated controls (67%).	[82]
TLK1B associated with rAAV9	AAV2/ 9-TLK1B groups showed no decline in salivary flow post-irradiation (121% increase) and salivary flow was not significantly different in irradiated and non-irradiated animals treated similarly with TLK1B.	[83]

Table 2.
Radioprotection of salivary glands, in vivo *using murine models.*

RABBIT		
Agent	**Main Findings**	**Ref**
Lidocaine HydroChloride	Pre-treatment with lidocaine improved irradiation tolerance of both, parotid and submandibular glands. Ultra-structure was largely preserved.	[84]
Lidocaine Amifostine Pilocarpin	Only animals pre-treated with lidocaine or amifostine (alone or combined with pilocarpin) showed a slight non-significant reduction in the salivary ejection fraction. Lidocaine and amifostine could largely preserve the glandular ultra-structure.	[85]
mini-PIG		
Agent	**Main Findings**	**Ref**
Orciprenaline Carbachol	Acinar cells of both glands were significantly more numerous in the pre-treatment group. Also, cells seemed better preserved. Yet, such effects were more pronounced in the parotid gland (appearing almost normal) than in the submandibular gland.	[86]
Adenoviral vector encoding FGF2 (AdLTR2EF1a-FGF2)	A single pre-administration of a hybrid serotype 5 adenoviral vector encoding FGF2 (AdLTR2EF1a-FGF2) resulted in the protection of parotid microvascular endothelial cells from irradiation damage and significantly limited the decline of parotid salivary flow.	[87]

Table 3.
Radioprotection of salivary glands, in vivo *using non-murine models.*

systems, with a look onto potential reparative and regenerative keys, where we, amongst other clinicians and researchers, do aspire for a superior, safe, efficacious and long-term innovative solution that reverses RT-induced damage to the salivary glands of our HNC patients. Moreover, we opted to avoid concluding our *overview* with calls for additional research or validation, given that vital tissue engineering strategies employing the design, characterization and optimization of novel biomaterials (and 3D printing), that can also be housing/incorporating release-controlled nanoparticles or nanocapsules that also are designed to encapsulate distinct mesenchymal stem cells, induced pluripotent stem cells (iPSCs), growth factors or cytokines and/or pharmaceutical agents or drugs, currently investigated at different levels of development are limitless in distinctions and details.

Palliative care for RT-induced salivary gland dysfunction- current and commercially-available palliative options for HNC patients undergoing RT include chewing gum (sugar-free), saliva substitutes, oral and topical lubricants, malic and ascorbic acid, saliva stimulants and sialogogue such as pilocarpine (Salagen, for

Agent	Main Findings	Ref
WR-2721	Administration of WR-2721 prior to each dose of irradiation was feasible and without significant toxicity at 100 mg/m^2. Salivary gland function improved over time after completion of RT, particularly in the parotid gland.	[88]
Botulinum Toxin A (BTX-A)	The SUVmean of the ^{225}Ac-labeled PSMA radio-ligand in the injected parotid gland (right) showed a highly significant decrease of up to 60% when compared with the left side in the 63 years old patient with advanced metastatic castration-resistant prostate cancer (suffering from sialorrhoea) receiving 80 units of BTX-A.	[89]
Amifostine	Amifostine reduces acute xerostomia and mucositis.	[90]
SMGT+IMRT	Surgical submandibular gland transfer (SMGT) was combined with intensity-modulated radiotherapy (IMRT) in a prospective phase II feasibility trial, in a single institution, including 40 HNC patients. At 12 months post-RT, the rate of absent or only mild xerostomia was 89%, and salivary flow rates were approximately 75% of pre-RT levels. Hence, patients reported decreased xerostomia and improved QoL.	[91]
Helical Tomotherapy (HT)	HT is described as an innovate, more precise and less toxic RT technique using a continuously rotating gantry to integrate 3D image guidance (a linear accelerator with computerized tomography) and deliver IMRT in a helical pattern. In 175 HNC patients, followed for up to 36 months, HT was used to deliver irradiation doses to bi-lateral parotid glands (PG-T), contra-lateral submandibular gland (cSMG), and accessory salivary glands in the oral cavity. Xerostomia was significantly decreased when the mean doses of PG-T, cSMG, and OC were kept below 29.12Gy, 29.29Gy, and 31.44Gy, respectively.	[92]

Table 4.
Radioprotection of salivary glands, clinically in human subjects.

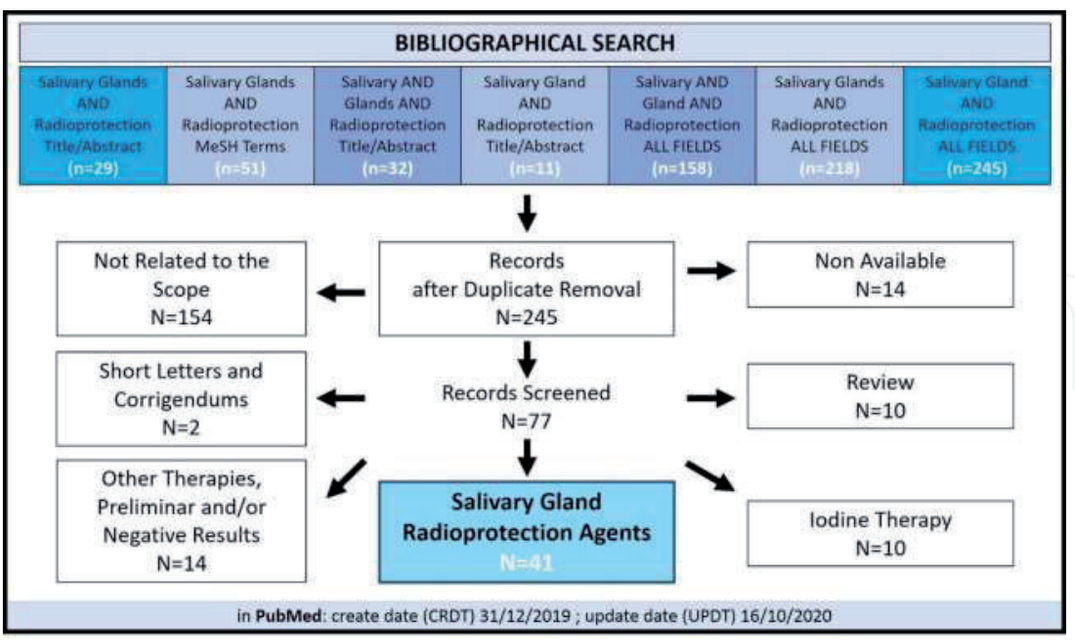


Figure 3.
PRISMA flow diagram for the bibliographic electronic search on PubMed central.

example) and cevimeline (Evoxac, for example). As mentioned above, none have proved to restore normal QoL and patient satisfaction, mainly due to their limited efficacy and effectiveness [30, 42]. On top, adverse side effects are common, and such options are often costly to patients, requiring multiple daily use over long

periods of time. In parallel, patients, especially the elderly, institutionalized and frail, need to go through education and training to acquire new eating and life-style habits, learn to prevent or avoid impaired swallowing and potential choking, and improve their oral and dental hygiene practices and tools to prevent (or halt the progression of) dental and oral mucosal diseases, infections and tooth loss. Other palliative care options including acupuncture and electro-stimulation (enhancement of salivary reflexes) are currently undergoing investigation [30, 93].

The only Food and Drug Administration–approved radioprotective and anti-xerostomia drug for clinical use (adjuvant setting) is Amifostine, an organic thiophosphate, cryoprotective agent and free radical scavenger administered subcutaneously or most often intravenously upon reconstitution with normal saline prior to or simultaneously with RT to then accumulate within the salivary glands, has been extensively-studied since its development, initially under the nuclear warfare program [14, 94]. Today, while it continues to benefit some patients, prophylactically, via minimizing the effects of xerostomia and taste loss, it is often associated with severe side effects including a rapid decrease in blood pressure (hypotension), nausea and emesis or vomiting. Recent analysis of several clinical trials associated Amifostine to low-quality and mixed evidence in preventing dry mouth complaints in patients receiving RT to the head and neck region, in the short- to medium-terms (up to three months post-RT) and have questioned its potential in tumor cell protection, thereby further narrowing its clinical safety and efficacy window, especially in light of its high cost [94, 95]. Essentially, its use in radiation-induced xerostomia has already been cautioned in the year 2008 by the American Society of Clinical Oncology [96], and so, its controversial and debatable safety and use in all cancer cases lingers.

Preventive and interventional care for RT-induced salivary gland dysfunction- the main objective of any planned and/or prescribed option should be the relief of symptoms and complications associated with hypo-salivation and xerostomia in HNC patients scheduled to receive RT, in order to prevent deteriorations in their QoL thereby enhancing their battle with cancer, its treatment and consequences [97]. As discussed earlier, despite advancements in irradiation techniques and regimens including IMRT, only palliative and prophylactic options are available, all of which do suffer substantial short-comings [98, 99]. One might even consider IMPT or intensity modulated proton therapy, used to deliver a much-reduced irradiation dose and subsequently less toxic than IMRT, thereby alleviating much of the typical side effects of RT, however, IMPT is known to be more expensive and lacks accessibility and availability [43, 98, 99].

1. **Surgical Intervention alternative**- to prevent RT-induced hyposalivation, sub-mandibular gland preservation and protection from irradiation via surgical relocation to the sub-mental space, thereby away or out of irradiation zone, has been explored, with positive results. It is perhaps worth mentioning herein that sub-mandibular salivary gland supplies up to 90% of the un-stimulated saliva formation/secretion. However, such highly-invasive interventional procedures are peculiar and require exquisite surgical manipulation skills and settings. Further, surgical transfer of salivary glands is not indicated or possible for cancers of the oral cavity or patients undergoing (systemic) chemotherapy. In addition, for the gland to either retain or restore functionality, the connection of the gland to the main duct must be maintained or restored, respectively [100], altogether render it a very limit-ed/—ing option.

   In terms of innovative approaches, Rao *et al.* [101] recently described the use of a synthetic hydrogel (TraceIT, composed of water and iodinated cross-linked polyethylene glycol), injected via an 18-gauge needle, to serve as a

minimally-invasive "spacer" (previously demonstrated in the treatment of prostate cancer), and displace or relocate the sub-mandibular gland in order to protect it from irradiation toxicity and be able to deliver a reduced irradiation dose, however the experimental model used comprised of four refrigerated cadaveric specimens and no further *in vivo* or clinical studies evaluated usability, malleability, safety and efficacy, amongst other factors, in clinical organ spacing.

2. **Tissue Engineering and Regenerative Medicine alternative**- clearly, better approaches need to be explored and developed, driving the search elsewhere, into the multi-disciplinary areas of tissue engineering and regenerative medicine, in order to combine with and improve current options or to innovate and translate new alternative solutions, for wound healing. This is especially true, in light of accumulating knowledge and understanding of the underlying mechanisms governing radiation-induced salivary gland damage and dysfunction [47]. Indeed, from inducing DNA damage (via: a. the generation of ROS/reactive oxygen species or b. the breakage of the DNA double strand), to mutations to cell death (by apoptosis or necrosis, depending on cell type, injury and cellular responses), to the loss of salivary progenitors, to the accruing evidence regarding the regenerative capacity (slow yet existent) of salivary glands following RT-induced injury, more evidently upon the administration of a stimuli (exogenous delivery of stem cells and/or growth factors, for example), altogether re-emphasize the potential of such complex yet innovative approaches in finding a better clinical alternative solution.

   In a recent *clinical* study, Ho *et al.* [102] evaluated the effects of a commercially-available slowly-dissolving adhering disc/tablet formulation (OraCoat XyliMelts) on the oro-dental health, enamel remineralization, bio-film formation, saliva presence, pH and buffering in 5 patients diagnosed with xerostomia (criteria: un-stimulated whole saliva flow rate below 0.2 mL per minute and a stimulated saliva flow rate of less than 0.5 mL in 5 minutes). They also assessed patient self-reported comfort with the mint-flavored, xylitol-releasing tablets. Subjects were instructed to use the disc as often as needed for dry mouth symptoms relief. At the end, a mean of 4 + 1 discs each day and 2 discs each night, were used. Overall, desirable effects of the product on symptomatic alleviation and management of xerostomia were reported. The authors reported effective local palliation, reduced dental sensitivity, improved salivary production and buffering capacity, reduced plaque formation and alleviated xerostomia symptoms, without the need to use any systemic sialagogue medications throughout the 21 days of the study [102]. Yet, this is a pilot study, limited for involving a small of number of participants.

Biomaterials and Cell Therapy- one of the fundamental roles for the maintenance of the body of any living organism is regeneration, which enables the repair and restoration of lost or damaged tissue [47, 103]. Adult stem/stromal and progenitor cells have been identified in many tissues, and are known to have a key role in the regeneration and repair, initiated or activated either by the excessive loss of differentiated cells (pool) or via (niche) environmental cues. In the presence of functional biomaterials such as the previously-described injectable hydrogel spacer [101] and a feasible agent-delivery tablet or disc [102], *would loading, encapsulating or incorporating putative salivary progenitor or stem/stromal cells, for example, a distinct type of stimuli, yield better results?* Supplying salivary gland progenitor and stem/stromal cells, via a proper release-controlled dose-responsive carrier, might be able to re-establish the disrupted salivary stem/progenitor cell pool and niche,

restore glandular tissue homeostasis, reverse hypo-salivation, and perhaps control xerostomia, a hypothesis we are currently examining in our laboratory, employing natural and synthetic polymers, liposomes, solid lipid nanoparticles and core-shell nanocapsules, and further supplementing by other pharmaceutical agents.

Modern medicine and biomedical research aim to control and enhance radio-protective as well as regenerative and reparative capabilities through the utilization of cells (cell lineages or primary cells), growing surface control using bio-scaffolds and/or manipulating growth factor/cytokine concentrations [47, 104], strategies designed to stimulate residual cells to regenerate acini and other parenchymal elements (ductal ligation) and infiltrate growth factor doses to boost salivary gland repair post-RT [105].

Growth Factor Therapy- somatomedin C is a hormone, similar to insulin in molecular structure, and actually is better known as IGF-1 or insulin-like growth factor 1 [106]. While a statement as "increased insulin-like growth factor signaling induces cell proliferation, survival and cancer progression" is true, it is traditional and partial, to a great extent. Today we understand that the issue is much more complex. For instance, IGF regulates cellular senescence which is known to halt proliferation of aged and stressed cells and do play a key role against cancer development. Actually, there is accruing evidence that, over time, IGF not only regulates but also induces pre-mature cellular senescence (tumor suppressor protein p53-dependant, in terms of acetylation, stabilization and activation) [107]. Hence, despite the *understandably-alarming, at first and for some, suggestion* to exogenously administer/supply cytokines and growth factors to sites of cancer, the recent years have indeed witnessed a note-worthy increase in the study of growth factors as cytoprotectants including their use as radioprotectors for salivary glands, and to reduce RT-induced symptoms, such as oral mucositis. To date, various growth factors have emerged as potential radioprotectors, including neurotrophic factors [108, 109], epidermal growth factor (EGF), fibroblast growth factor (FGF) [51, 110], keratinocyte growth factor (KGF) [111, 112] and the afore-mentioned insulin-like growth factor-1 or IGF-1 [55, 113, 114]. Meyer *et al.,* [113], for example, investigated and determined the radioprotectant and therapeutic effect of IGF-1, in a murine model. They found that IGF-1 is mediated by the activation and maintenance of a histone deacetylase, specifically the Sirtuin 1 (SirT-1). Pre-treatment with IGF-1 enabled the repair of double-stranded breaks in the DNA of parotid salivary gland cells within the first hours post-irradiation, thereby allowing for optimal DNA repair (*i.e.* IGF-1 promotes DNA repair in irradiated parotid salivary glands via the maintenance and activation of SirT-1) to fulfill the cell cycle checkpoints. However, hours later and as early as 8 h, RT-induced apoptotic cells were detected [113]. Such observations lead to further study the signaling cross-talk between IGF-1 and SirT-1, thereby identifying several activators, stabilizers and inhibitors, including the afore-mentioned inhibition of the p53-mediated apoptosis and the phosphoinositide 3-kinase (PI3K) – protein kinase B (Akt) pathway [107], in-depth study-worthy topics, beyond the scope of this concise review. To date, studies, collectively indicate that cytokines can be radioprotective, anti-apoptotic and suggest/promote that the exogenous and localized (via a release-controlled delivery system, preferably directly injectable) utilization of growth factors do stimulate endogenous stem cell populations/niche and will eventually contribute to the desired and/or pursued clinical solution suitable for preventing RT-induced damage, diminishing salivary hypofunction, as well as restoring salivary gland function in irradiated HNC cases.

Gene Transfer Therapy- the utilization of gene transfer, DNA transmission and cell transduction to produce high levels of transgenic protein in order to correct cellular dysfunction and/or induce a new cellular function, post-RT, is a wide area of investigation and development. Baum *et al.* [115], utilized an adenoviral technique to transfer the Aquaporin-1 (AQP1) gene into the sub-mandibular gland, reporting

an increase in salivary flow when compared to control viruses into rat or mini-pig models [115, 116]. Yet, key shortcomings continue to exist for non-viral as well as viral vectors [103], rendering translation for routine clinical use difficult. Likewise, the therapeutic potential of genetic modification and application of small-interfering RNAs or siRNA for the purpose of target gene silencing are intensively investigated, progressing from pre-clinical testing in animal models to ongoing clinical trials for cancer, lung disease and liver damage in human subjects. Thus far, highly limited in salivary gland tissues and accompanied with significant safety concerns [50]. For example, AQP-1 gene transfer into the salivary glands via adeno-viral vectors to treat disorders such as SS, yielded strong immune responses, mainly due to the limited or low efficiency of intra-cellular siRNA delivery [117, 118]. Herein, similar to growth factors, cell therapy and pharmaceutical agent administration, the availability of a reproducible, scalable, safe and effective, release-controlled carrier/vehicle suitable for therapeutic siRNA delivery, directly into the salivary gland, ensuring sufficient residency/retention, is a challenge.

5. Closing remarks

5.1 Wnt/β-catenin pathway: radio-protective role and effect in RT-induced salivary gland damage

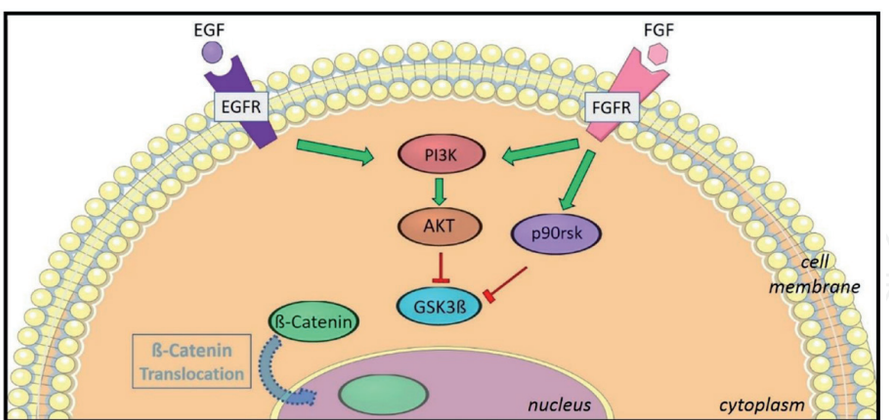
In irradiation studies and radioprotection literature, numerous cellular signaling pathways and cell-cycle alteration mechanisms have been explored. Of those, the Wnt/β-catenin signaling pathway seems to receive the utmost attention, recently, towards preventing the damage caused by irradiation [119]. Briefly, this canonical Wingless–Int (Wnt) pathway leads to the accumulation and translocation of co-activator β-catenin, a multi-functional protein involved in cell–cell adhesion, gene transcription and physiologic homeostasis (adullt), into the nucleus, via a series of molecular events initiated through the binding of specific Wnt proteins to the frizzled receptors on the cell surface. The pathway plays a critical role in cell regulating cell migration and determining cell fate, and mutations have been linked to human birth defects, cancer and other disorders and diseases [120–123].

Activating the canonical Wnt/β-catenin signaling pathway is complex. It depends on a family of glyco-proteins involved in cell-to-cell communication. To simplify, the interaction of ß-catenin with the cell adhesion molecule, e-cadherin, is involved in phenotypes: adhesion, mobility and proliferation [121, 122]. In absence of a Wnt ligand, β-catenin is degraded by the "destruction complex". Several proteins are involved within this complex whereby Axin acts as a scaffold protein facilitating the interaction of Glycogen Synthase Kinase 3β (GSK-3β), Adenomatous Polyposis Coli (APC) and Casein Kinase 1α (CK1α), for β-catenin phosphorylation [123, 124]. Then, phosphorylated β-catenin is recognized by the β-transducin-repeat-containing protein (β-TrCP) and goes through the ubiquitin-proteasome degradation pathway. When the Wnt ligand activates Wnt signaling through the plasmatic membrane receptor frizzled with other lipoprotein receptors, the cytoplasmic protein dishevelled (Dvl) is recruited and thereby activated. Herein, the activation of Dvl disrupts the "destruction complex" by dissociation of the GSK-3β from the Axin and inhibits the GSK-3β. As a result, β-catenin phosphorylation is also inhibited, allowing stabilization and translocation of β-catenin into the nucleus. Nuclear β-catenin then binds to a transcription factor-T cell factor and a lymphoid-enhancing factor (Tcf/Lef) and finally activates a response, *i.e.* changes in gene expression [120, 125, 126].

The Wnt signaling pathway cross-talks with other signaling pathways, and can be modulated by several activators and inhibitors. For example, the utilization of

growth factors, to activate or inhibit, has been extensively studied, further adding to the complexity given the wide range of involved genes [119]. Cross-talk between signaling pathways is possible via the common regulatory protein GSK-3β. For example, when the epidermal growth factor (EGF) is recognized by its native receptor (EGF-R), this complex activates the afore-mentioned phosphoinositide 3-kinase (PI3K) which facilitates the activation of AKT kinase regulator. Herein, the activation of AKT results in the inhibition of GSK-3β by phosphorylation [127–129] and ultimately leads to the translocation of β-catenin into the nucleus. On the other hand, the fibroblast growth factor (FGF) is also able to cross-talk with GSK-3β (common pathway with EGF) and the activation of its native receptor (FGF-R) is followed by PI3K which then results in the inhibition of GSK-3β via AKT activation [125, 130]. Herein, FGF-R activation also involves MapK activation which inhibits GSK-3β through the p90 ribosomal protein s6 kinase (p90rsk) in an AKT-independent manner [131–133]. Therefore, activating the Wnt signaling pathway (**Figure 4**) through the utilization of cytoplasmic regulatory proteins (from other signaling pathways) is potentially able to promote β-catenin stabilization, its translocation to the nucleus and the activation of survival genes [134]. Such understanding and revelations can lead to produce a plausible and innovative alternative strategy for the activation of native repair systems that may allow and promote the survival of the cells during and after RT. Possibly, can be even extended to explore plausibility for prevention.

To the best of knowledge, Hakim *et al.* [135] conducted one of the first/earliest *clinical* studies connecting signaling pathways (Wnt/β-catenin and TGF-β) with salivary gland irradiation damage. They reported an alteration in the expression pattern of Wnt1 in viable irradiated acinar cells of xerostomic patients, suggesting a possible therapeutic effect of the Wnt pathway in controlling RT-induced salivary gland damage and dysfunction [135], in accordance with previous *in vitro* studies [120]. Following this line of research, Hai *et al.* [136] carried out a study analyzing the transient activation of the Wnt/β-catenin signaling pathway to prevent



Cross-talk between key elements of the EGF-, FGF- and Wnt-pathways, illustrating how the activation of cell membrane receptors (EGFR and FGFR) regulates (+/-) the Wnt pathway. In the depicted mechanism, activation of either the PI3-Kinase/AKT or the p90rsk (MAP Kinase) can promote the phosphorylation of GSK-3ß leading to the *nuclear translocation* of the multi-functional protein **ß-Catenin** from the cytoplasm.

Figure 4.
EGF and FGF pathway(s) interaction with ß-catenin and canonical Wnt signaling pathway.

irradiation damage to the salivary glands. They reported, using a murine model, that activating the Wnt/β-catenin pathway through the transient activation of Wnt1 in the basal epithelium helped to prevent chronic salivary dysfunction generated by local irradiation, specifically via suppressing apoptosis and preserving or rescuing the life of salivary stem/progenitor cells. Salivation in experimental mice when compared to controls (animals receiving only RT) was increased/higher [120, 136]. However, the radioprotective effect of Wnt/β-catenin activation seems, thus far, to only occur within a limited time lapse. Activating the signaling path 3 days before or 3 days after irradiation yielded dissimilar effects on the tissues [136].

Indeed, in another approach, the activation and modulation of cell signaling pathway(s) using a cocktail (more than one) of activators has been suggested, with the Wnt signaling pathway (and its components) as therapeutic target(s). Thula *et al.* [51] evaluated the effect of EGF and bFGF (basic FGF) in salivary gland explants, reporting promising results regarding gland radioprotection [51]. Overall, taking the studied findings into account, it can be proposed that a Wnt/β-catenin signaling pathway activator might be a good candidate to be developed as a potential preventive and therapeutic strategy against the RT-induced salivary gland damage. Herein, as was and is the present scenario with cells, proteins, genes, growth factors and drugs, a suitable delivery vehicle is once more, deemed vital.

Technology Promise in Translational Tissue Engineering and NanoMedicine- the interplay between tissue engineering, regenerative medicine, biomaterials, bio-nanotechnology and nanomedicine continues to be the hallmark of current scientific research World-wide, promising to change every aspect of human life via creating revolutionary materials of biological origin for use in the diagnosis and treatment of devastating human diseases, a multi-disciplinary approach to innovative and translational solutions, suitable for scale-up, safe, efficacious and cost-effective routine clinical use [137–139]. Whether conventional small-molecule agents or emerging protein and/or peptide-based macromolecular biopharmaceutics, therapeutic effect is of vital significance. Controlled or at least predictable delivery is also substantially necessary. An intense effort is invested into engineering such complex bio-systems capable to achieve optimum cell-material interactions, while keeping intact the materials bulk properties. One of the core interests of nanobiotechnology, for example, this decade has been drug/gene/cell bio-functional delivery, driving the design and development of bio-inspired, intelligent or "smart" nano-systems [137, 138, 140]. It can be stated that a competitive and superiorly successful delivery system should offer: therapeutic outcome enhancement, patient compliance improvement and overall cost reduction of therapy. For HNC cases suffering RT-induced salivary gland damage and dysfunction, an attractive delivery system, for clinical ease-of-use, can perhaps entail a directly injectable formulation, sterilizable, capable to efficiently-hold a dose-responsive bio-load, maintain its bio-activity over time, and "predictably" control its pharmaco-kinetic release profile.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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