DOI: https://doi.org/10.54393/pjhs.v5i12.1755

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (P): 2790-9352, (E): 2790-9344
Volume 5, Issue 12 (December 2024)

Systematic Review

OPEN ACCESS

Cellular and Molecular Mechanisms of Salivary Gland Development and Regeneration: Implications for Tissue Engineering and Regenerative Medicine

Zain-Ud-Din Ahmed[1], Maheen Rao[1], Fatima Jawad[2], Safi Ullah Khan[3*], Taha Rehman[4] and Majida Rahim[5]

[1]Akhtar Saeed Medical and Dental College, Lahore, Pakistan
[2]Department of Pathology, Al-Aleem Medical College, Lahore, Pakistan
[3]Department of Oral Biology, Multan Medical and Dental College, Multan, Pakistan
[4]Department of Oral and Maxillofacial Surgery, Armed Forces Institute of Dentistry, Rawalpindi, Pakistan
[5]Department of Oral and Maxillofacial Surgery, Foundation University School of Health Sciences, Islamabad, Pakistan

ARTICLE INFO

Keywords:
Regenerative Medicine, Stems Cells, Tissue Engineering, 3D Tissue Culturing, Artificial Salivary Glands

How to Cite:
Ahmed, Z.-U.-D., Rao, M., Jawad, F., Khan, S. U., Rehman, T., & Rahim, M. (2024). Cellular and Molecular Mechanisms of Salivary Gland Development and Regeneration: Implications for Tissue Engineering and Regenerative Medicine: Salivary Gland Development and Regeneration. Pakistan Journal of Health Sciences, 5(12). https://doi.org/10.54393/pjhs.v5i12.1755

***Corresponding Author:**
Safi Ullah Khan
Department of Oral Biology, Multan Medical and Dental College, Multan, Pakistan
safiullah1990.sk@gmail.com

Received date: 20th May, 2024
Accepted date: 18th December, 2024
Published date: 31st December, 2024

ABSTRACT

Salivary glands are essential for oral health, but their function can be compromised by cancer, autoimmune disorders, infections, and physical traumas, severely impacting quality of life. There is currently no cure for salivary gland dysfunction, and treatment is symptomatic. **Objective:** To explore the cellular and molecular mechanisms involved in the development, maturation, and regeneration of salivary glands, with a focus on tissue engineering and regenerative medicine. **Methods:** A comprehensive review was conducted using PRISMA and information was fetched through PUBMED, EMBASE, Medline, and Google Scholar databases. **Results:** The FGF pathway, part of the growth factor family, plays a significant role in salivary gland homeostasis, while the Wnt pathway is crucial for gland maturation. Various receptors and signaling molecules are involved in the gland's functioning. Recent advancements in regenerative medicine have demonstrated that activating endogenous stem cells can lead to positive outcomes in restoring injured salivary glands. Technological advancements in 3D tissue culturing using patient cells have enabled the creation of functional artificial salivary gland organs. However, no cell line completely mimics natural salivary gland cells, and their inherent tumorigenic potential delays their therapeutic application. **Conclusions:** Understanding these mechanisms is vital for developing effective therapies. While recent advancements show promise, further research is necessary to create safe, accurate cell lines for therapeutic use. This knowledge is crucial for establishing therapeutic avenues that could potentially lead to direct regeneration, reconstruction, and replacement of functioning salivary glands.

INTRODUCTION

The Salivary Glands (SGs) perform pivotal actions required for the sustenance of oral health. Dysfunction in these glands can lead to significant deterioration in oral functioning and the emergence of other health issues [1]. The parotid, submandibular, sublingual, and numerous minor salivary glands secrete saliva in response to a variety of biochemical signals and environmental stimuli [2]. Saliva, composed of water, mucus, antimicrobial substances, electrolytes, and a variety of enzymes, is essential for speaking, eating, swallowing, digestion, and maintaining the health of teeth and gingival tissues [3]. Irreversible damage to the SG secretion pathway results in hyposalivation, exhibited as xerostomia, or dry mouth, in individuals with autoimmune diseases like Sjögren's syndrome or those undergoing radiation therapies for head and neck tumors [4]. Globally, at least 3.1 million adults suffer from Sjögren's syndrome, predominantly affecting middle-aged and older women [5]. Additionally, radiation therapy for head and neck cancer impacts about 1 million new patients annually [6, 7]. Current treatments for hyposalivation are palliative and provide only temporary relief from xerostomia [8]. Therefore, re-engineering SGs could offer long-term, practical methods to restore normal salivation [9]. In 1999, Bruce Baum and colleagues identified three key strategies for re-engineering salivary epithelial cell functions: generating artificial SGs, mending

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

hypofunctional SGs, and redesigning secretory functions [10]. Since then, numerous tissue engineering techniques have been explored to restore salivation, with some progressing to clinical studies [11, 12]. Stem cell therapy, in combination with specific cell culture techniques such as scaffold materials, hanging drop and rotating culture vessels, and spontaneous cell aggregation has shown promise in clinical trials for developing functional secretory epithelial organs [13-15]. Gene therapies also offer novel treatment opportunities for radiation-induced xerostomia [16-18]. Despite these advancements, maintaining the effective secretory capacity of SG cells remains challenging. Researchers have explored the combination of various cell types and biomaterials to create the ideal implant material for SG tissue engineering [19, 20].

The objectives were to summarize the current body of knowledge regarding stem cell anatomy, structure, and function related to SGs. The review aims to summarize current knowledge on stem cell anatomy, structure, and function in relation to salivary glands (SGs), investigate stem cell pathologies and dysfunctions within SGs, and identify key components of tissue engineering strategies for SG regeneration.

METHODS

The systematic review adhered to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and explored cellular and molecular mechanisms in salivary gland development, maturation, and regeneration, with a focus on tissue engineering and regenerative medicine. Databases such as PUBMED, EMBASE, Medline, and Google Scholar were searched using terms like "Salivary gland," "Salivary gland maturation," and "Salivary gland regeneration." Inclusion criteria covered studies from 1995 to 2022 in English, while excluding studies on non-human salivary glands, pre-1995 publications, and non-English studies. The review process involved identification, screening, eligibility, and inclusion phases to address the research inquiry on molecular mechanisms relevant to salivary gland tissue engineering and regenerative medicine.

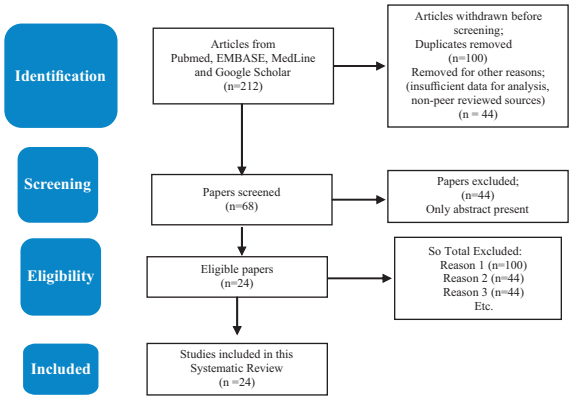

Figure 1: PRISMA flow-chart for studies selection

RESULTS

The information collected after reviewing the articles stated that SG function relies on a complex network of molecular pathways that regulate cellular homeostasis, growth, and response to injury. These key pathways, including Fibroblast Growth Factor (FGF), Wnt, Hedgehog (Hh), and Notch signaling, coordinate gland maturation, repair, and functional regeneration. This regulatory framework also includes specific growth factors and signaling molecules that guide cellular differentiation, maintain glandular structure, and support tissue engineering strategies for potential regenerative therapies. Salivary gland function is regulated by a complex network of molecular pathways. The Fibroblast Growth Factor (FGF) pathway is essential for maintaining gland homeostasis, with FGF-receptor 2 in intercalated and excretory duct cells and FGF-7 in salisphere-forming cells, indicating its trophic role. Another study reported that in Vivo Fibroblast Growth factor receptor-1 (FGFR1) and Fibroblast Growth factor receptor 2b and (FGFR2b) signaling are indispensable for SMG development. The Wnt pathway is essential in the maturation of salivary glands, playing roles during mesenchymal activation and the differentiation of the ductal epithelium and lumen development [21-23]. Mature salivary gland ductal epithelium maintains Wnt signaling expression, supporting regeneration after damage. This study explores the role of Wnt signaling in regulating salivary gland (SG) development, focusing on the balance between pro-acinar differentiation and duct formation. Wnt signaling is shown to maintain end bud cells in an undifferentiated state during early development by suppressing Proto-Oncogene Receptor Tyrosine Kinase (KIT) expression through the up regulation of Myb transcription factor, which inhibits the Phosphatidylinositol 3-Kinase - KT (PI3K-AKT) pathway [24]. This suggests that Wnt Signaling regulates SGs repair after wound injury. Hedgehog (Hh) signaling is triggered by branching morphogenesis and is inhibited when the ligand is blocked in vivo and in vitro [25]. Glioma-associated oncogene homolog 1 (Gli1), a Hh-target gene, promotes epithelial proliferation during functional regeneration, with overexpression linked to the reactivation of multiple salivary gland progenitors [26]. Hh signaling is vital for salivary gland tissue regeneration as it supports the preservation of functional salivary stem/progenitor cells and sustains parasympathetic innervation, both of which are critical for restoring gland function following damage, such as that caused by irradiation [27]. Gli1mediated Hedgehog signaling aids salivary gland regeneration by restoring function and macrophage interactions post-injury, with minimal involvement in fibrosis. Hh pathway activation, similar to SMG regeneration post-duct ligation, was confirmed in salisphere cultures. Hedgehog plays a critical role in regulating epithelial branching and salivary gland morphogenesis by positively interacting with Fgf8.

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

The Notch signaling pathway, involving target gene Hes1 and receptors NOTCH1 and NOTCH4, along with ligands JAGGED1, JAGGED2, and DELTA1, regulates growth and differentiation in adult murine salivary glands [28-30]. Notch signaling is active during regeneration after duct damage, crucial for maintaining SG tissue homeostasis [31]. Notch signaling directs salivary gland morphogenesis by promoting luminal cell differentiation from Krt8+ bipotent progenitors and restricting basal/myoepithelial fates. Its inhibition disrupts branching and epithelial organization, highlighting its essential role in lineage specification [32, 33]. Innervation is crucial for salivary gland (SG) function, with Glial cell line-Derived Neurotrophic Factor (GDNF) driving parasympathetic nerve formation and its genetic deletion reducing innervated acini. Nerve Growth factor (NGF), essential for sympathetic innervation, is concentrated in the murine submandibular gland, where it regulates cell survival, axonal development, angiogenesis, tissue remodeling, and wound healing through its high and low-affinity receptors [33]. In another study, Neural Cell Adhesion Molecule (NCAM) In the group with intact innervation, NCAM expression was significantly increased during the early stages of regeneration, suggesting importance of intact parasympathetic innervation in promoting ductal cell proliferation and overall regeneration of the submandibular gland [34]. The polarity of apical and basal cell regions is regulated by Int-3 and Int-1, while cell-extracellular matrix contact and branching morphogenesis depend on integrin receptor Int-6 and its interaction with Laminin-1. Int-6, Int-1, and Int-4 are found in multipotent cells in adult tissues. Autoimmune Sjögren's syndrome is characterized by low levels of Laminin-1 [35-37]. The α3β1 integrin is essential for the differentiation of salivary gland cells, with its absence leading to altered expression of important extracellular matrix (ECM) components and adhesion molecules, including laminin and E-cadherin [38]. Another study highlights the critical roles of β1 and β4 integrins in salivary gland development, particularly in cytodifferentiation and tissue organization, while β3 integrin is associated with vascular development and adult glands. Integrins are essential for gland morphogenesis and function [39]. In vivo outcomes on the role of Sonic Hedgehog (Shh) in salivary gland (SMG) development suggest that Shh is a critical downstream mediator of Ectodysplasin (Eda) signaling [40]. Shh Expression Correlates with Eda Levels Shh transcript levels in developing SMGs were found to correlate with Eda statuswhich in turn drives SMG branching morphogenesis [41]. Shh expression in Eda-null (Eda–/–) glands was lower than in wild-type and K14-Eda transgenic glands, suggesting other regulatory factors. Treatment with recombinant Shh increased branching in Eda–/– glands, had a weaker effect in control glands, and no effect in K14-Eda glands, indicating Eda modulates Shh's impact on branching. Both Shh and Eda proteins partially rescued the Eda–/– phenotype by increasing end bud numbers. Cyclopamine inhibition of hedgehog signaling reduced branching in control and K14-Eda glands but not in Eda–/– glands, highlighting dependence on Eda signaling. Shh was mainly localized in the epithelium of developing SMGs, with higher expression in K14-Eda glands and reduced in Eda–/– glands [42]. This suggests that, Eda is a key regulator of Shh which induces branching morphogenesis of SGs. The differential effects of recombinant Shh and Cyclopaminealso suggest that Eda act as modulator on how Shh influences branching morphogenesis. SGdysfunction can lead to hyposalivation (xerostomia) or hypersalivation (sialorrhea). Xerostomia affects at least 10% of adults, with higher prevalence in women and the elderly [43]. Sialorrhea may result from primary SG modifications, drug side effects, or neurological diseases [44]. Saliva is crucial for food processing, tooth protection, and microbial defense [45]. Mucosal alterations and infections predispose individuals to chronic SG hypofunction [46]. Drug side effects are common causes of SG hypofunction, especially in older adults [46, 47]. SG inflammation (sialadenitis) is often linked to hyposalivation and duct blockage [48]. Sjögren's syndrome, affecting 0.1-4.8% of the population, is a chronic autoimmune condition with a 9:1 female to male ratio [49, 50]. Symptoms include dry mouth and dry eyes [51]. Radiation therapy for head and neck cancer affects a large number globally, leading to irreversible SG damage [52]. Remaining SG regeneration capacity after radiation may offer new therapeutic avenues [53]. Current xerostomia treatments, including stimulant drugs, secretagogues, salivary replacements, or artificial saliva, provide only temporary relief [54]. Gene therapy, stem cell therapy and SG bioengineered models are considered promising for restoring SG function. Tissue engineering for SG regeneration involves combining cells, bioactive substances, and biomaterials [55-59]. Cells are essential for SG regeneration, with single cells or those preserving 3D spatial arrangement used for implantation [60, 61]. Flow cytometry or selective enhancement during in vitro growth divides single cells into subpopulations of parenchymal and stromal cells [62]. The challenge lies in combining SG stem/progenitor cells with biocompatible, biodegradable scaffolds, ensuring cell survival and function for therapeutic relevance [63]. Stem cells are a type of immature cell which possesses an astonishing capacity to regenerate and repair any tissue or organ in the body owing to their unique ability to proliferate, differentiate, and renew themselves [64]. Stem cell therapy is a type of tissue engineering that helps to develop biological substitutes that can restore, maintain, or improve the function of damaged tissues or organs [65]. It is reported that tissue aging and structural changes are primary reasons for SG dysfunction. Genetic tracing in animal models identified progenitor cells in mature SGs. Acini produce new acinar cells post-damage, not ductal cells, indicating an innate fate-commitment program [66, 67]. Sex-determining

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

Region Y box gene (SOX2)-labeled acinar cells can develop into Mucin 19 (MUC19)expressing acinar cells after radiation [15]. SOX2 expression in adult major SGs suggests its use for regeneration [68]. In vitro stem cell expansion systems, like submandibular gland-derived stem cell culture, enhance the pool of functionally committed cells [69]. Radiation exposure impacts stem cells, which can be effectively transplanted in vivo. This study investigated the role of androgens in salivary gland remodeling, focusing on their effects on laminin α1 chain and integrin (INT) α1 and α2 subunit expression in human salivary gland cells and labial salivary gland (LSG) tissues from healthy individuals and patients with Sjögren's Syndrome (SS). Androgens such as DHEA and testosterone increased INT α1 and α2 subunit mRNA and protein levels in intercalated duct and acinar cells from healthy individuals, while laminin α1-chain expression remained unaffected. However, this androgen-driven upregulation was absent in SS LSG, suggesting defective androgen regulation in SS contributes to impaired outside-in signaling, acinar cell atrophy, and ductal cell hyperplasia, hallmark features of the disease [70]. This suggested thatSOX2-positive cells are a promising target for regenerating damaged salivary glands, especially after injury caused by radiation. Kit-expressing cells are located in peripheral epithelial end-bud cells of the Submandibular Gland Keratin 14 (Krt14) expressing progenitors are influenced by Kit signaling and FGFr2b, with Krt5+ epithelial progenitors preserving the neuronal niche [71]. Krt5 progenitors in mature glands are mainly restricted to the SMG duct. KIT and KRT14 mark a shared progenitor population in the SG that plays a critical role in ductal system maturation and granulated duct (GD) formation during development. The dynamic expression of KRT14 and its later restriction to junctional regions imply a maintained progenitor-like state in these cells, potentially supporting long-term SG homeostasis and regeneration [72]. Mesenchymal progenitor cells in healthy adult SGs multiply locally in response to wounds, regenerating both acini and ducts [73]. ASCL3-expressing ductal cells produce both ductal and acinar cells Krt5-progenitors persist in smaller glands after Ascl3+-population elimination [74, 75]. These findings indicated that multiple progenitor populations in SGs capable of compensating for each other's losses. Bi-dimensional tissue cultures lack cell-to-cell and cell-to-extracellular matrix interactions suggesting that the microenvironment's impact on cell renewal, proliferation, and differentiation is crucial. Modeling diseases requires considering the altered microenvironment to understand disease complexity. Advancements in 3D tissue culturing have enabled the creation of functional artificial salivary glands using bioengineered templates with cell-seeded scaffolds, replicating structural complexity for research. While primary cells have limited growth and lifespan, techniques using cell lines offer promise, though no cell line fully replicates natural SG cells, hindering therapeutic progress [43, 73, 75].

Table 1: Summary of Salivary Gland Molecular Pathways and their role in possible Regenerative Strategies

Molecular Pathway	Function/Role	Summary of Study Findings
FGF Pathway	Maintains Salivary Gland homeostasis; Fibroblast Growth Factor-receptor 2 in duct cells, Fibroblast Growth factor 7 in saliosphere-forming cells. These findings suggest that Fibroblast growth factor and fibroblast receptors regular branching morphogenesis	In vivo, FGF-2 exhibited strong immunostaining in intercalated ducts and blood vessels initially, while FGF-7 appeared on Day 0, peaked on Days 3 and 7, and vanished by Day 14 [21]. In vivo studies show FGFR1 and FGFR2b signaling are essential for SMG development, with knockout models highlighting their roles in morphogenesis and differentiation. In vitro findings reveal FGFR1 regulates branching morphogenesis through downstream FGFs and BMPs [22].
Wnt Pathway	Essential for mesenchymal activation, ductal epithelium differentiation, and lumen development	In vivo studies with β-Catenin -Activated Transgene galactosidase mice showed Wnt/β-catenin signaling activity in basal intercalated duct cells at postnatal day 1 and increased during SMG regeneration [23]. Wnt signaling regulates salivary gland development by maintaining end bud cells in an undifferentiated state, suppressing Proto-Oncogene Receptor Tyrosine Kinase (KIT) via Myb upregulation, and inhibiting the Phosphatidylinositol 3-Kinase-AKT (PI3K-AKT) pathway to delay proacinar differentiation [24].
Hedgehog (Hh) Signaling	Triggered by branching morphogenesis; Gli1 promotes epithelial proliferation, linked to progenitor reactivation	Hedgehog (Hh) signaling is triggered by branching morphogenesis and is inhibited when the ligand is blocked in vivo and in vitro [26].Hedgehog signaling supports salivary gland regeneration by preserving stem/progenitor cells and parasympathetic innervation essential for functional recovery [27]. These studies examined Hedgehog signaling in SMG regeneration using Krt5 -rtTA/tetO-Shh mice, showing significant upregulation of Hh target genes in male mice after doxycyclineinduction, while female mice exhibited a weaker response; techniquesincluded qPCR, X-gal staining, H&E staining, immunofluorescence,Western blot, spheroid culture, PCR Array for Wnt components, and RT-qPCR [28].

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

		Hedgehog plays a critical role in regulating epithelial branching and salivary gland morphogenesis by positively interacting with Fgf8. In Tabby mice, Shh downregulation due to EdaTa mutation disrupts branching, partially compensated by upregulation of Egf/Tgfα/Egfr pathways [29].
Notch Signaling	Regulates growth and differentiation; active during regeneration post-duct damage	The study examined Notch signalingin salivary gland development, showing that Human Salivary Gland cells expressedNotch receptors and ligands, with differentiation markers upregulated on Matrigel and requiring γ-secretase for activation. In vivo studies demonstrated intenseNotch signaling in ductal and regenerating acinar cells, highlighting its essential role in glandular cell differentiation and injury recovery [30].
GDNF and NGF Signaling	GDNF for parasympathetic innervation, NGF for sympathetic innervation; NGF controls cell survival and angiogenesis	In the murine submandibular gland,essential for sympathetic innervation, it regulates cell survival, axonal growth, angiogenesis, remodeling, and healing via high- and low-affinity receptors [33].In this study, Neural Cell Adhesion Molecule (NCAM) In the group with intactinnervation,NCAM expression was significantly increased during the early stages of regeneration, suggesting importance of intact parasympathetic innervation in promoting ductal cell proliferation and overall regeneration of the submandibular gland [34]. The study showed reduced trkAand p75NTR protein levels and sympathetic axon density in NGF+/– mice, impacting glandular innervation. Despite no significant change in NGF levels in sub mandibular salivary glands, pineal glands exhibited higher NGFvariability, indicating effects on sympathetic neuron survival [35]. Glial cell line-derived neurotrophic factor (GDNF) promotes the survival and proliferation of salivary gland stem cells, especially following irradiation, through activation of the GDNF-RET signaling pathway [36].
Integrins and Laminin1	Int-3, Int-1 for polarity; Int-6 and Laminin1 for ECM contact and branching morphogenesis	3β1 integrin deficiency in embryonic SMGs causes basement membrane defects, acinar disorganization, and mucin increase, while Lama5 deficiency disrupts epithelial organization and delays development by E17.5 [37]. Another study reports that α3β1 integrin is crucial for salivary gland cell differentiation, with its absence disrupting ECM components like laminin and adhesion molecules such as E-cadherin [38].Another study shows β1 and β4 integrins drive salivary gland development, while β3 supports vascular development, underscoring their role in morphogenesis [39].
Shh expression and its correlation with Eda levels	Regulates Shh expression, promotes branching morphogenesis and inhibition of Hh signaling	A study suggested that Shh is critical downstream mediator of Ectodysplasin (Eda) signaling [40].In another study it was reported that Eda signaling isa key regulator of Shh,which in turn drives SMG branching morphogenesis [41]. Another study found that Shh transcript levels were reduced but not absent in Eda–/– glands, suggesting involvement of additional regulatory factors. Recombinant Shh increased branching in Eda–/– gland showing Eda-dependent Shh signaling in salivary gland development [42].Kit signaling cells influence keratin 14gene which after injury helps in regenerating both acini and ducts [53].
Sjögren's Syndrome and Radiation	Characterized by low Laminin-1, androgens (integrin α1 and α2) autoimmune epithelitis, and radiation-induced Salivary gland damage	The findings of these studies suggested SOX2 nerve-dependent mechanism are playing important role for regenerating damaged salivary glands, especially after injury caused by radiation [68].In vitro study findings suggested that submandibular gland's stems cells that provide potential for regeneration [69].Androgens increased integrin α1 and α2 subunits in salivary gland cells and healthy tissues but not in Sjögren's syndrome SS glands, where defective regulation contributes to acinar atrophy and ductal hyperplasia [70]. In another study found that KIT and KRT14 shared progenitor population in the salivary glandthat plays a critical role in ductal system maturation during development. Suggesting that it supports Salivary gland homeostasis and regeneration.Potentially playing an important role in regeneration post radiation [72].

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

DISCUSSION

The study of salivary gland tissue engineering is advancing rapidly due to its impact on oral health and quality of life. This review explores molecular mechanisms, pathologies, and regenerative strategies for restoring salivary gland function, highlighting current challenges and future directions in the field [26, 27]. Understanding the molecular pathways involved in salivary gland development, maturation, and regeneration is essential for effective therapeutic interventions. The review highlights key pathways such as FGF, Wnt, Hedgehog (Hh), and Notch signaling are crucial for salivary gland homeostasis, repair, and regeneration [77-79]. The interplay of these pathways is crucial for cellular differentiation, proliferation, and function, essential for maintaining SG integrity. Dysregulation can lead to SG disorders, highlighting the need for targeted molecular therapies to restore normal function [80]. The high prevalence of SG dysfunction, especially xerostomia, highlights the need for effective treatments. Conditions like Sjögren's syndrome and radiation-induced damage lead to chronic dry mouth, with current treatments being mainly palliative. This review emphasizes the importance of developing regenerative therapies to address the underlying causes of SG dysfunction [81]. Tissue engineering for SG regeneration combines cells, bioactive substances, and biomaterials to create functional substitutes. The review highlights the promise of stem cell therapies, especially SG-derived stem/progenitor cells, and advances in scaffold materials and 3D culturing techniques for developing functional SG models [82, 83]. Gene therapy represents another innovative approach, particularly for addressing radiation-induced SG damage [84]. Gene therapies targeting specific molecular pathways could restore normal salivation by enhancing the regenerative capacity of salivary gland cells. Additionally, biomimetic models and 3D tissue cultures are valuable for studying salivary gland function and disease, offering insights into how the microenvironment influences cellular behavior and regeneration [85, 86]. These models can also facilitate the development of personalized medicine approaches; tailoring treatments to individual patients' needs. Despite progress, challenges in SG tissue engineering include cellular differentiation, scaffold design, and tissue integration. Future research must refine technologies, explore novel biomaterials, and optimize stem cell and gene therapy protocols, ensuring safety and efficacy through rigorous testing.

CONCLUSIONS

This systematic review highlighted the potential of stem cell and gene therapies in salivary gland (SG) tissue engineering, emphasizing the need for targeted approaches to restore SG function. It outlines molecular mechanisms underlying SG development and regeneration. Continued research promises effective, long-term solutions for SG-related conditions, enhancing oral health and quality of life.

Authors Contribution

Conceptualization: ZUDA
Methodology: MR, FJ, ZUDA
Formal analysis: MR
Writing, review and editing: SUK, TR

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Pillai S, Munguia-Lopez JG, Tran SD. Bioengineered Salivary Gland Microtissues— A Review of 3D Cellular Models and their Applications. American Chemical Society Applied Bio Materials. 2024 Apr; 7(5): 2620-36. doi: 10.1021/acsabm.4c00028.

[2] Almansoori AA, Kim B, Lee JH, Tran SD. Tissue engineering of oral mucosa and salivary gland: disease modeling and clinical applications. Micromachines.2020 Nov; 11(12): 1066. doi: 10.3 390/ mi11121066.

[3] Rose C and Olley RC. Patient preventive advice to mitigate signs and symptoms of tooth wear. Dental Update.2024Jun;51(6):422-6.doi:10.1296 8/denu. 2024.51.6.422.

[4] Villa A, Wolff A, Narayana N, Dawes C, Aframian DJ, Lynge Pedersen AM *et al.* World Workshop on Oral Medicine VI:a systematic review of medication-induced salivary gland dysfunction. Oral Diseases.2016Jul;22(5):365-82. doi: 10.1111/ odi.12402.

[5] Chery G. Understanding Sjögren's Syndrome as a Systemic Autoimmune Disorder. State University of New York at Albany; 2022.

[6] Ferreira JN, Rungarunlert S, Urkasemsin G, Adine C, Souza GR. Three-dimensional bioprinting nanotechnologies towards clinical application of stem cells and their secretome in salivary gland regeneration.Stem Cells International.2016 Dec;

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

2016(1): 7564689. doi: 10.1155/2016/7564689.

[7] Mercadante V, Jensen SB, Smith DK, Bohlke K, Bauman J, Brennan MT *et al*. Salivary gland hypofunction and/or xerostomia induced by nonsurgical cancer therapies: ISOO/MASCC/ASCO guideline. Journal of Clinical Oncology.2021 Sep;39 (25): 2825-43. doi: 10.1200/JCO.21.01208.

[8] Parisis D, Chivasso C, Perret J, Soyfoo MS, Delporte C. Current state of knowledge on primary Sjögren's syndrome, an autoimmune exocrinopathy. Journal of Clinical Medicine.2020 Jul; 9(7): 2299. doi: 10.3390/jcm9072299.

[9] Seo YJ, Lilliu MA, Abu Elghanam G, Nguyen TT, Liu Y, Lee JC *et al.* Cell culture of differentiated human salivary epithelial cells in a serum-free and scalable suspension system: The salivary functional units model. Journal of Tissue Engineering and Regenerative Medicine.2019 Sep; 13(9): 1559-70. doi: 10.1002/term.2908.

[10] Baum BJ, Wang S, Cukierman E, Delporte C, Kagami H, Marmary Y *et al.* Re-engineering the functions of a terminally differentiated epithelial cell in vivo. Annals of the New York Academy of Sciences. 1999 Jun; 875(1): 294-300.doi: 10.1111/j.1749-6632.1999.tb08512.x

[11] Barrows CM, Wu D, Farach-Carson MC, Young S. Building a functional salivary gland for cell-based therapy: more than secretory epithelial acini. Tissue Engineering Part A. 2020 Dec; 26(23-24): 1332-48. doi: 10.1089/ten.tea.2020.0184.

[12] Blitzer GC, Rogus-Pulia NM, Mattison RJ, Varghese T, Ganz O, Chappell R *et al.* Marrow-Derived Autologous Stromal Cells for the Restoration of Salivary Hypofunction (MARSH): Study protocol for a phase 1 dose-escalation trial of patients with xerostomia after radiation therapy for head and neck cancer: MARSH: Marrow-Derived Autologous Stromal Cells for the Restoration of Salivary Hypofunction. Cytotherapy. 2022 May; 24(5): 534-43. doi: 10.1016/j.jcyt.2021.11.003.

[13] Adine C, Ng KK, Rungarunlert S, Souza GR, Ferreira JN. Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting for stimulating epithelial growth in salivary glands. Biomaterials.2018 Oct; 180: 52-66. doi: 10.1016/j.biomaterials.2018.06.011.

[14] Nam K, Wang CS, Maruyama CL, Lei P, Andreadis ST, Baker OJ. L1 peptide-conjugated fibrin hydrogels promote salivary gland regeneration. Journal of Dental Research. 2017 Jul; 96(7): 798-806. doi: 10.1177/0022034517695496.

[15] Lombaert IM, Brunsting JF, Wierenga PK, Faber H, Stokman MA, Kok T *et al.* Rescue of salivary gland function after stem cell transplantation in irradiated glands. PLOS One. 2008 Apr; 3(4): e2063. doi: 10.1371/journal.pone.0002063.

[16] Nanduri LS, Lombaert IM, Van Der Zwaag M, Faber H, Brunsting JF, Van Os RP *et al.* Salisphere derived c-Kit+ cell transplantation restores tissue homeostasis in irradiated salivary gland. Radiotherapy and Oncology. 2013 Sep; 108(3): 458-63. doi: 10.1016/j.radonc.2013.05.020.

[17] Grønhøj C, Jensen DH, Vester-Glowinski P, Jensen SB, Bardow A, Oliveri RS *et al.* Safety and efficacy of mesenchymal stem cells for radiation-induced xerostomia: a randomized, placebo-controlled phase 1/2 trial (MESRIX). International Journal of Radiation Oncology* Biology* Physics.2018 Jul; 101(3): 581-92. doi: 10.1016/j.ijrobp.2018.02.034.

[18] Lynggaard CD, Grønhøj C, Jensen SB, Christensen R, Specht L, Andersen E *et al.* Long-term safety of treatment with autologous mesenchymal stem cells in patients with radiation-induced xerostomia: primary results of the MESRIX phase I/II randomized trial. Clinical Cancer Research. 2022 Jul; 28(13): 2890-7. doi: 10.1158/1078-0432.CCR-21-4520.

[19] Ozdemir T, Fowler EW, Liu S, Harrington DA, Witt RL, Farach-Carson MC *et al.* Tuning hydrogel properties to promote the assembly of salivary gland spheroids in 3D. ACS Biomaterials Science & Engineering. 2016 Dec; 2(12): 2217-30. doi: 10.1021/acsbiomaterials.6b00419.

[20] Shubin AD, Felong TJ, Schutrum BE, Joe DS, Ovitt CE, Benoit DS. Encapsulation of primary salivary gland cells in enzymatically degradable poly(ethylene glycol) hydrogels promotes acinar cell characteristics. Acta Biomaterialia.2017 Mar; 50: 437-49. doi: 10.1016/j.actbio.2016.12.049.

[21] Shimizu O, Yasumitsu T, Shiratsuchi H, Oka S, Watanabe T, Saito T *et al.* Immunolocalization of FGF-2,-7,-8,-10 and FGFR-1-4 during regeneration of the rat submandibular gland. Journal of Molecular Histology. 2015 Oct; 46: 421-9. doi: 10.1007/s10735-015-9631-6.

[22] Hoffman MP, Kidder BL, Steinberg ZL, Lakhani S, Ho S, Kleinman HK *et al.* Gene expression profiles of mouse submandibular gland development: FGFR1 regulates branching morphogenesis in vitro through BMP-and FGF-dependent mechanisms.2002 Dec; 129(24): 5767-78. doi: 10.1242/dev.00172.

[23] Hai B, Yang Z, Millar SE, Choi YS, Taketo MM, Nagy A, Liu F. Wnt/β-catenin signaling regulates postnatal development and regeneration of the salivary gland. Stem cells and development. 2010 Nov 1;19(11):1793-

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

801. doi: 10.1089/scd.2009.0499

[24] Matsumoto S, Kurimoto T, Taketo MM, Fujii S, Kikuchi A. The WNT/MYB pathway suppresses KIT expression to control the timing of salivary proacinar differentiation and duct formation. Development. 2016 Jul; 143(13): 2311-24. doi: 10.1242/dev.134486.

[25] Hu L, Du C, Yang Z, Yang Y, Zhu Z, Shan Z et al. Transient activation of hedgehog signaling inhibits cellular senescence and inflammation in radiated swine salivary glands through preserving resident macrophages. International Journal of Molecular Sciences.2021 Dec; 22(24): 13493. doi: 10.3390 /ijms222413493.

[26] Wells KL, Mou C, Headon DJ, Tucker AS. Recombinant EDA or Sonic Hedgehog rescue the branching defect in Ectodysplasin A pathway mutant salivary glands in vitro. Developmental Dynamics.2010 Oct; 239(10): 267 4-84. doi: 10.1002/dvdy.22406

[27] Zhao Q, Zhang L, Hai B, Wang J, Baetge CL, Deveau MA *et al.* Transient activation of the Hedgehog-Gli pathway rescues radiotherapy-induced dry mouth via recovering salivary gland resident macrophages. Cancer Research. 2020 Dec; 80(24): 5531-42. doi: 10.1158/0008-5472.CAN-20-0503.

[28] Hai B, Qin L, Yang Z, Zhao Q, Shangguan L, Ti X *et al.* Transient activation of hedgehog pathway rescued irradiation-induced hyposalivation by preserving salivary stem/progenitor cells and parasympathetic innervation. Clinical Cancer Research. 2014 Jan; 20(1): 140-50. doi: 10.1158/1078-0432.CCR-13-1434.

[29] Melnick M, Phair RD, Lapidot SA, Jaskoll T. Salivary gland branching morphogenesis: a quantitative systems analysis of the Eda/Edar/NFκB paradigm. BioMed Central Developmental Biology. 2009 Dec; 9: 1-22. doi: 10.1186/1471-213X-9-32.

[30] Dang H, Lin AL, Zhang B, Zhang HM, Katz MS, Yeh CK. Role for Notch signaling in salivary acinar cell growth and differentiation. Developmental dynamics: an official publication of the American Association of Anatomists.2009 Mar; 238(3): 724-31. doi: 10.100 2/dvdy.21875.

[31] Imatani A and Callahan R. Identification of a novel NOTCH-4/INT-3 RNA species encoding an activated gene product in certain human tumor cell lines. Oncogene. 2000 Jan; 19(2): 223-31. doi: 10.103 8/sj.onc.1203295.

[32] Mikkola ML. Molecular aspects of hypohidrotic ectodermal dysplasia. American Journal of Medical Genetics Part A.2009 Sep; 149(9): 2031-6. doi: 10.100 2/ajmg.a.32855.

[33] Schenck K, Schreurs O, Hayashi K, Helgeland K. The role of nerve growth factor (NGF) and its precursor forms in oral wound healing. International Journal of Molecular Sciences.2017 Feb; 18(2): 386. doi: 10.339 0/ijms18020386.

[34] Wang X, Li Z, Shao Q, Zhang C, Wang J, Han Z *et al.* The intact parasympathetic nerve promotes submandibular gland regeneration through ductal cell proliferation. Cell Proliferation. 2021 Jul; 54(7): e13078. doi: 10.1111/cpr.13078.

[35] Næsse EP, Schreurs O, Messelt E, Hayashi K, Schenck K. Distribution of nerve growth factor, pro-nerve growth factor, and their receptors in human salivary glands. European Journal of Oral Sciences. 2013 Feb; 121(1): 13-20. doi: 10.1111/eos.12008.

[36] Peng X, Varendi K, Maimets M, Andressoo JO, Coppes RP. Role of glial-cell-derived neurotrophic factor in salivary gland stem cell response to irradiation. Radiotherapy and Oncology.2017 Sep; 124(3): 448-54. doi: 10.1016/j.radonc.2017.07.008.

[37] Kadoya YU and Yamashina SH. Distribution of alpha 6 integrin subunit in developing mouse submandibular gland. Journal of Histochemistry & Cytochemistry. 1993 Nov; 41(11): 1707-14. doi: 10.1177/41.11.8409377.

[38] Menko AS, Kreidberg JA, Ryan TT, Van Bockstaele E, Kukuruzinska MA. Loss of α3β1 integrin function results in an altered differentiation program in the mouse submandibular gland. Developmental dynamics: an official publication of the American Association of Anatomists. 2001 Apr; 220(4): 337-49. doi: 10.1002/dvdy.1114.

[39] Lourenço SV and Kapas S. Integrin expression in developing human salivary glands. Histochemistry and Cell Biology. 2005 Nov; 124: 391-9. doi: 10.1007/ s00418-005-0784-3.

[40] Pummila M, Fliniaux I, Jaatinen R, James MJ, Laurikkala J, Schneider P *et al.* Ectodysplasin has a dual role in ectodermal organogenesis: inhibition of Bmp activity and induction of Shh expression. 2007 Jan; 134(1): 117-25. doi: 10.1242/dev.02708.

[41] Häärä O, Fujimori S, Schmidt-Ullrich R, Hartmann C, Thesleff I, Mikkola ML. Ectodysplasin and Wnt pathways are required for salivary gland branching morphogenesis. Development. 2011 Jul; 138(13): 2681-91. doi: 10.1242/dev.057711.

[42] Wells KL, Mou C, Headon DJ, Tucker AS. Recombinant EDA or Sonic Hedgehog rescue the branching defect in Ectodysplasin A pathway mutant salivary glands in vitro. Developmental Dynamics.2010 Oct; 239(10): 267 4-84. doi: 10.1002/dvdy.22406.

[43] Chen Z, Chen X, Zhu B, Yu H, Bao X, Hou Y *et al.* TGF-β1 Triggers Salivary Hypofunction via Attenuating Protein Secretion and AQP5 Expression in Human Submandibular Gland Cells.Journal of Proteome

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

Research. 2023 Aug; 22(9): 2803-13. doi: 10.102 1/acs.jproteome.3c00052.
[44] Porcheri C and Mitsiadis TA. Physiology, pathology and regeneration of salivary glands. Cells. 2019 Aug; 8(9): 976. doi: 10.3390/cells8090976.
[45] Emmerson E, Knox SM. Salivary gland stem cells: a review of development, regeneration and cancer. genesis. 2018; 56(5): e23211. doi: 10.1002/dvg.23211.
[46] Holmberg KV and Hoffman MP. Anatomy, biogenesis and regeneration of salivary glands. Saliva: secretion and functions.2014 May; 24: 1-3. doi: 10.1159/ 000 358776.
[47] Pedersen AM, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. Journal of Oral Rehabilitation. 2018 Sep; 45(9): 730-46. doi: 10.1111/joor.12664.
[48] Gervais EM, Sequeira SJ, Wang W, Abraham S, Kim JH, Leonard D *et al.* Par-1b is required for morphogenesis and differentiation of myoepithelial cells during salivary gland development. Organogenesis. 2016 Oct; 12(4): 194-216. doi: 10.1080/15476278.2016.1252887.
[49] Wu D, Chapela PJ, Barrows CM, Harrington DA, Carson DD, Witt RL *et al.* MUC1 and polarity markers INADL and SCRIB identify salivary ductal cells. Journal of Dental Research.2022 Jul; 101(8): 983-91. doi: 10.1177/0022 0 345221076122.
[50] Brazen B and Dyer J. Histology, salivary glands. 2019.
[51] Proctor GB and Carpenter GH. Salivary secretion: mechanism and neural regulation. Saliva: Secretion and functions. 2014; 24: 14-29. doi: 10.1159/000358781.
[52] Khalafalla MG, Woods LT, Jasmer KJ, Forti KM, Camden JM, Jensen JL *et al.* P2 receptors as therapeutic targets in the salivary gland: from physiology to dysfunction. Frontiers in Pharmacology. 2020 Mar; 11: 222. doi: 10.3389/fphar.2020.00222.
[53] Rugel-Stahl A, Elliott ME, Ovitt CE. Ascl3 marks adult progenitor cells of the mouse salivary gland. Stem Cell Research.2012 May; 8(3): 379-87. doi: 10.1016/j .scr .2012.01.002.
[54] Brosky ME. The role of saliva in oral health: strategies for prevention and management of xerostomia. The Journal of Supportive Oncology. 2007 May; 5(5): 215-25.
[55] Smith CH, Boland B, Daureeawoo Y, Donaldson E, Small K, Tuomainen J. Effect of aging on stimulated salivary flow in adults. Journal of the American Geriatrics Society. 2013 May; 61(5): 805-8. doi: 10.1111/jgs.12219.
[56] Tanaka J and Mishima K. Application of regenerative medicine to salivary gland hypofunction. Japanese Dental Science Review. 2021 Nov; 57: 54-9. doi: 10.10 16/j.jdsr.2021.03.002.
[57] Nelson J, Manzella K, Baker O. Current cell models for bioengineering a salivary gland: a mini-review of emerging technologies. Oral Diseases. 2013 Apr; 19(3): 236-44. doi: 10.1111/j.1601-0825.2012.01958.x.
[58] Jaskoll T, Leo T, Witcher D, Ormestad M, Astorga J, Bringas Jr P *et al.* Sonic hedgehog signaling plays an essential role during embryonic salivary gland epithelial branching morphogenesis. Developmental dynamics: an official publication of the American Association of Anatomists. 2004 Apr; 229(4): 722-32. doi: 10.1002/dvdy.10472.
[59] Bolk K, Mueller K, Phalke N, Walvekar RR. Management of Benign salivary gland conditions. Surgical Clinics. 2022 Apr;102(2): 209-31. doi: 10.1016/j. suc.2022. 01.001.
[60] Tzioufas AG and Voulgarelis M. Update on Sjögren's syndrome autoimmune epithelitis: from classification to increased neoplasias. Best Practice & Research Clinical Rheumatology. 2007 Dec; 21(6): 989-1010. doi: 10.1016/j.berh.2007.09.001.
[61] Pynnonen MA, Gillespie MB, Roman B, Rosenfeld RM, Tunkel DE, Bontempo L *et al.* Clinical practice guideline: evaluation of the neck mass in adults. Otolaryngology-Head and Neck Surgery.2017 Sep; 157 (2): 30. doi: 10.1177/0194599817722550.
[62] Luitje ME, Israel AK, Cummings MA, Giampoli EJ, Allen PD, Newlands SD *et al.* Long-term maintenance of acinar cells in human submandibular glands after radiation therapy. International Journal of Radiation Oncology* Biology* Physics. 2021 Mar; 109(4): 1028-39. doi: 10.1016/j.ijrobp.2020.10.037.
[63] Baum BJ, Alevizos I, Chiorini JA, Cotrim AP, Zheng C. Advances in salivary gland gene therapy-oral and systemic implications. Expert Opinion on Biological Therapy.2015 Oct; 15(10): 1443-54. doi: 10.1517/14712 598. 2015.1064894.
[64] Donnelly H, Salmeron-Sanchez M, Dalby MJ. Designing stem cell niches for differentiation and self-renewal. Journal of the Royal Society Interface. 2018 Aug; 15(145): 20180388. doi: 10.1098/rsif.2018.0388.
[65] Mousaei Ghasroldasht M, Seok J, Park HS, Liakath Ali FB, Al-Hendy A. Stem cell therapy: from idea to clinical practice. International Journal of Molecular Sciences. 2022 Mar; 23(5): 2850. doi: 10.3390/ijms23052850.
[66] Emmerson E, May AJ, Berthoin L, Cruz-Pacheco N, Nathan S, Mattingly AJ *et al.* Salivary glands regenerate after radiation injury through SOX2-mediated secretory cell replacement. European Molecular Biology Organization Molecular Medicine.2018 Mar; 10(3): e8051. doi: 10.15 252/emmm. 201708051.

DOI: https://doi.org/10.54393/pjhs.v5i12.1755

[67] Feng J, van der Zwaag M, Stokman MA, van Os R, Coppes RP. Isolation and characterization of human salivary gland cells for stem cell transplantation to reduce radiation-induced hyposalivation. Radiotherapy and Oncology.2009 Sep; 92(3): 466-71. doi: 10.1016/j.radonc.2009.06.023.

[68] Ninche N, Kwak M, Ghazizadeh S. Diverse epithelial cell populations contribute to the regeneration of secretory units in injured salivary glands. Development.2020 Oct; 147(19): dev192807. doi: 10.1101/2020.06. 29.177733.

[69] Laine M, Virtanen I, Salo T, Konttinen YT. Segment-specific but pathologic laminin isoform profiles in human labial salivary glands of patients with Sjögren's syndrome. Arthritis & Rheumatism.2004 Dec; 50(12): 3968-73. doi: 10.1002/art.20730.

[70] Porola P, Laine M, Virtanen I, Pöllänen R, Przybyla BD, Konttinen YT. Androgens and integrins in salivary glands in Sjögren's syndrome. The Journal of Rheumatology. 2010 Jun; 37(6): 1181-7. doi: 10.389 9/jrheum.091354.

[71] Bullard T, Koek L, Roztocil E, Kingsley PD, Mirels L, Ovitt CE. Ascl3 expression marks a progenitor population of both acinar and ductal cells in mouse salivary glands. Developmental Biology. 2008 Aug; 320 (1): 72-8. doi: 10.1016/j.ydbio.2008.04.018.

[72] May AJ, Cruz-Pacheco N, Emmerson E, Gaylord EA, Seidel K, Nathan S *et al*. Diverse progenitor cells preserve salivary gland ductal architecture after radiation-induced damage. Development.2018 Nov; 145(21): dev166363. doi: 10.1242/dev.166363.

[73] Matheu A, Collado M, Wise C, Manterola L, Cekaite L, Tye AJ *et al*. Oncogenicity of the developmental transcription factor Sox9. Cancer Research. 2012 Mar; 72(5): 1301-15. doi: 10.1158/0008-5472.CAN-11-3660.

[74] Ivanov SV, Panaccione A, Nonaka D, Prasad ML, Boyd KL, Brown B *et al*. Diagnostic SOX10 gene signatures in salivary adenoid cystic and breast basal-like carcinomas. British Journal of Cancer. 2013 Jul; 109(2): 444-51. doi: 10.1038/bjc.2013.326.

[75] Redman RS. On approaches to the functional restoration of salivary glands damaged by radiation therapy for head and neck cancer, with a review of related aspects of salivary gland morphology and development. Biotechnic & Histochemistry. 2008 Jan; 83(3-4): 103-30. doi: 10.1080/10520290802374683.

[76] Häärä O, Fujimori S, Schmidt-Ullrich R, Hartmann C, Thesleff I, Mikkola ML. Ectodysplasin and Wnt pathways are required for salivary gland branching morphogenesis. Development. 2011 Jul; 138(13): 2681-91. doi: 10.1242/dev.057711.

[77] Wells KL, Mou C, Headon DJ, Tucker AS. Recombinant EDA or Sonic Hedgehog rescue the branching defect in Ectodysplasin A pathway mutant salivary glands in vitro. Developmental Dynamics.2010 Oct; 239(10): 2674-84. doi: 10.1002/dvdy.22406.

[78] Borkosky SS, Nagatsuka H, Orita Y, Tsujigiwa H, Yoshinobu J, Gunduz M *et al*. Sequential expressions of Notch1, Jagged2 and Math1 in molar tooth germ of mouse. Biocell. 2008 Dec; 32(3): 251-7. doi: 10.326 04/biocell.2008.32.251.

[79] Mitsiadis TA, Graf D, Luder H, Gridley T, Bluteau G. BMPs and FGFs target Notch signalling via jagged 2 to regulate tooth morphogenesis and cytodifferentiation. Development. 2010 Sep; 137(18): 3025-35. doi: 10.1242/dev.049528.

[80] Qu J, Song M, Xie J, Huang XY, Hu XM, Gan RH *et al*. Notch2 signaling contributes to cell growth, invasion, and migration in salivary adenoid cystic carcinoma. Molecular and Cellular Biochemistry. 2016 Jan; 411: 135-41. doi: 10.1007/s11010-015-2575-z.

[81] Błochowiak K. Co-Existence of Dry Mouth, Xerostomia, and Focal Lymphocytic Sialadenitis in Patients with Sjögren's Syndrome. Applied Sciences. 2024 Jun; 14(13): 5451. doi: 10.3390/app14135451.

[82] Chibly AM, Aure MH, Patel VN, Hoffman MP. Salivary gland function, development, and regeneration. Physiological reviews. 2022 Jul; 102(3): 1495-552. doi: 10.1152/physrev.00015.2021.

[83] Hajiabbas M, D'Agostino C, Simińska-Stanny J, Tran SD, Shavandi A, Delporte C. Bioengineering in salivary gland regeneration. Journal of Biomedical Science. 2022 Jun; 29(1): 35. doi: 10.1186/s12929-022-00819-w.

[84] Aframian DJ amd Palmon A. Current status of the development of an artificial salivary gland. Tissue Engineering Part B: Reviews. 2008 Jun; 14(2): 187-98. doi: 10.1089/ten.teb.2008.0044.

[85] Sui Y, Zhang S, Li Y, Zhang X, Hu W, Feng Y *et al*. Generation of functional salivary gland tissue from human submandibular gland stem/progenitor cells. Stem Cell Research & Therapy. 2020 Dec; 11: 1-3. doi: 10.1186/s13287-020-01628-4.

[86] Wang T, Huang Q, Rao Z, Liu F, Su X, Zhai X *et al*. Injectable decellularized extracellular matrix hydrogel promotes salivary gland regeneration via endogenous stem cell recruitment and suppression of fibrogenesis. Acta Biomaterialia. 2023 Oct; 169: 256-72. doi: 10.1016/j.actbio.2023.08.003.