Journal of Oral Biology and Craniofacial Research 13 (2023) 693–703

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Extracellular matrix turnover in salivary gland disorders and regenerative therapies: Obstacles and opportunities

Milos Marinkovic [a,b], Olivia N. Tran [a], Hanzhou Wang [a], Parveez Abdul-Azees [a,b], David D. Dean [a,c], Xiao-Dong Chen [a,b,c,**], Chih-Ko Yeh [a,d,*]

[a] Department of Comprehensive Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX, 78229-3900, USA
[b] Research Service, South Texas Veterans Health Care System, San Antonio, TX, 78229-4404, USA
[c] Department of Biomedical Engineering, University of Texas at San Antonio, San Antonio, TX, 78249, USA
[d] Geriatric Research, Education and Clinical Center, South Texas Veterans Health Care System, San Antonio, TX, 78229-4404, USA

ARTICLE INFO

Keywords:
Salivary gland
Aging
Matrix metalloproteinases
Extracellular matrix
Tissue remodeling
Fibrosis

ABSTRACT

Salivary gland (SG) extracellular matrix (ECM) has a major influence on tissue development, homeostasis, and tissue regeneration after injury. During aging, disease, and physical insult, normal remodeling of the SG microenvironment (i.e. ECM) becomes dysregulated, leading to alterations in matrix composition which disrupt tissue architecture/structure, alter cell activity, and negatively impact gland function. Matrix metalloproteinases (MMPs) are a large and diverse family of metalloendopeptidases which play a major role in matrix degradation and are intimately involved in regulating development and cell function; dysregulation of these enzymes leads to the production of a fibrotic matrix. In the SG this altered fibrotic ECM (or cell microenvironment) negatively impacts normal cell function and the effectiveness of gene and stem cell therapies which serve as a foundation for many SG regenerative therapies. For this reason, prospective regenerative strategies should prioritize the maintenance and/or restoration of a healthy SG ECM. Mesenchymal stem cells (MSCs) have great potential for mitigating damage to the SG microenvironment by ameliorating inflammation, reducing fibrosis, and repairing the damaged milieu of extracellular regulatory cues, including the matrix. This review addresses our current understanding of the impact of aging and disease on the SG microenvironment and suggests critical deficiencies and opportunities in ECM-targeted therapeutic interventions.

1. Introduction

The extracellular matrix (ECM) contains a multitude of biochemical, physical/mechanical, and architectural cues which regulate virtually all aspects of cellular function.[1–3] As in many other tissues, salivary gland (SG) contains a specific microenvironment, composed of regulatory cues embedded in the ECM, that direct normal cell turnover and tissue regeneration in response to injury. While studies of SG dysfunction have largely focused on the role of particular cell types, alterations in cell function are also suggestive of significant changes in the regulatory microenvironment. Although different types of SG dysfunction are likely associated with a unique set of changes in microenvironmental cues, ECM remodeling emerges as a consistent theme.[4]

The ECM is a highly dynamic and complex structure that undergoes controlled and continual remodeling in each tissue. Accordingly, alterations in the biochemical composition (i.e. proteins, complex polysaccharides, lipids, etc) of the SG ECM has attracted much attention as an important aspect in both disease pathogenesis and strategies for regeneration.[5] Under homeostatic conditions, ECM remodeling is a tightly regulated process, which involves the coordinated action of matrix metalloproteinases (MMPs) and their endogenous inhibitors (i.e. tissue inhibitors of metalloproteinases [TIMP]), as well as other families of ECM-modifying enzymes (e.g. serine, cysteine, and aspartic acid proteinases).[2,6] However, in aging or disease, ECM remodeling is

* Corresponding author. Department of Comprehensive Dentistry, University of Texas Health Science Center at San Antonio 7703 Floyd Curl Drive San Antonio, Texas 78229, USA.
** Corresponding author. Department of Comprehensive Dentistry University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX, 78229, USA.
E-mail addresses: marinkovic@uthscsa.edu (M. Marinkovic), olivianguyentran@gmail.com (O.N. Tran), wangh5@uthscsa.edu (H. Wang), abdulazees@uthscsa.edu (P. Abdul-Azees), deand@uthscsa.edu (D.D. Dean), chenx4@uthscsa.edu (X.-D. Chen), yeh@uthscsa.edu (C.-K. Yeh).

https://doi.org/10.1016/j.jobcr.2023.08.009
Received 12 June 2023; Accepted 28 August 2023

dysregulated and can lead to the accumulation of abnormal amounts (and types) of ECM components which disrupt tissue architecture and normal cell function.[7,8] On the other hand, fibrosis is a process that replaces normal/healthy ECM with disorganized scar tissue. In SG, fibrotic degeneration occurs as a natural consequence of aging, but is exacerbated in response to injury (e.g. irradiation) or chronic inflammation (e.g. Sjögren's syndrome [SS]).[9] The accumulation of fibrotic ECM in the SG microenvironment impairs glandular function by disrupting the normal architecture of the tissue, reducing the availability of nutrients and oxygen to the cells, and creating a barrier to the infiltration of progenitors that are required for regeneration.

Aberrant cues in the local microenvironment (i.e. the SG-ECM) have not been targeted for repair by cell-based tissue engineering approaches or prospective gene therapies. If left unaddressed, the damaged microenvironment provides an opposing influence which negates the potential therapeutic effects of the intervention. For example, aquaporin-1 (*AQP1*) gene therapy increases transitory expression of aquaporin-1 by SG cells and may improve saliva production to some extent, but it does not restore depolarization of the acinar cells and address the reduction in secretory activity caused by MMP-mediated breakdown of the ECM.[10] Thus, communication (i.e. crosstalk) between discrete molecular or gene-based interventions (e.g. *AQP1* gene therapy) and changes in signaling cues in the damaged microenvironment (e.g. tissue disorganization) impede efforts to restore SG function. In another words, the vast majority of current strategies for treating SG disorders focus on modulating cell proliferation, differentiation and function without any consideration for the damaged microenvironment (e.g., ECM) where the cells reside. In our view, it is essential that any new therapeutic paradigm carefully consider the precise mechanisms by which cues in the SG ECM regulate tissue homeostasis and how degradation or damage of this matrix contributes to SG dysfunction.[10–12] The aim of this review is to summarize our current understanding in this area and synthesize information in order to suggest novel approaches for mitigating or even reversing SG degeneration.

2. MMP dysregulation is linked to pathological remodeling of the SG ECM

MMPs are a 28-member family of zinc- and calcium-dependent endopeptidases that catabolize ECM components during matrix remodeling. In the SG, MMPs play a critical role in regulating extracellular cues during SG development, maintaining tissue homeostasis, and are involved in a variety of disease processes.[13,14] During SG development, MMPs remodel the ECM in order to support the growth and increasing complexity of the gland during morphogenesis. For example, MMP-2, -9, -14, and −15 are involved in orchestrating branching morphogenesis by degrading the basement membrane to support duct extension.[15] Additionally, MMP-3 and MMP-7 are involved in the differentiation of SG cells by cleaving cell surface receptors and modulating signaling pathways.

In human SGs, the expression of MMP-2 and -9, along with TIMP-1, -2, and -3, has been identified in mature ductal cells of healthy adults.[16,17] During SG homeostasis, MMPs are involved in ECM turnover, allowing for proper gland function. For example, MMP-2, -3, and -14 are involved in the maintenance of gland structure by regulating basement membrane turnover.[18] In contrast, when MMP activity is not appropriately regulated, changes in the microenvironment are associated with many forms of SG damage, such as gland degeneration during aging, ionizing radiation (IR)-induced injury, and autoimmune attack in SS.[19] The activity of MMPs is regulated by a variety of mechanisms, including the presence of endogenous inhibitors such as TIMPs, which help regulate ECM turnover by binding active MMPs to inhibit their activity. While all of the factors which regulate the balance of MMPs to TIMPs in the SG are yet to be elucidated, the expression of inflammatory cytokines such as interleukins (IL) -1α and −6, tumor necrosis factor (TNF)-α, and interferon-γ have been shown to induce the expression of MMPs/TIMPs in human SG cells.[20,21] Our current understanding of the role of MMPs in mediating the changes occurring in the SG microenvironment during physiological aging, IR-induced damage, and SS are summarized in Fig. 1.

2.1. SG degeneration during aging

Breakdown of the SG basal lamina, which occurs in aging, contributes to acinar cell depolarization and a reduction in secretory function. However, the mechanisms involved in SG degeneration with aging are more diverse than those implicated in discrete SG disease pathologies.[22] Biological variation between individuals, as well as a multitude of aging-related co-morbidities, increases the difficulty of isolating specific aging-related changes in human SGs. As a result, much of our current understanding of the aging SG microenvironment has been derived from animal models. An investigation into the structural and immunohistochemical changes in major SGs in young (2-months) and old (aging) (18-months) mice revealed an age-related increase in MMP-2 immunoreactivity.[23] In addition, Type I collagen immunoreactivity was notably elevated in aged mice and accompanied by morphological and functional deterioration of both acinar and ductal cells. Consistent with these findings, another study observed that acinar autolysis increases significantly with age.[24] Complimentary findings were also shown in a report which demonstrated that submandibular gland (SMG) from 24-month old mice exhibited significantly higher expression of MMP-2 compared to that of younger counterparts (3-months). In SMGs from aging mice, type I collagen and MMP-2 expression accumulated in the gland interstitium, suggesting an on-going fibrotic remodeling of the aging SG-ECM.[25]

A similar relationship between changes in the SG microenvironment and MMP expression has also been observed in human subjects enrolled in clinical studies of SG hypofunction and aging. Nassar et al. investigated SG biomarkers of aging in young (20–30 years) and elderly (60–80 years) patients.[26] The results showed a significant, aging-related reduction in salivary flow rate in whole saliva from the elderly versus young subjects. In addition, the saliva of elderly patients contained a higher concentration of MMP-1 and MMP-8, without a compensatory increase in TIMP-1 production. Interestingly, Holmström et al. reported that saliva levels of MMP-12, which is responsible for elastin proteolysis, were significantly decreased in older patients as compared to younger counterparts (<40 years old).[27] Together, these studies support the assertion that alterations in endogenous matrix remodeling activity are associated with aging-related changes in SG morphology and microenvironmental cues, independent of any specific pathology (e.g. SS).

As primary agents of matrix remodeling, MMPs play an important role in disrupting SG tissue architecture during aging. However, the mechanisms underlying the increased expression of MMPs in SGs during aging are still not well-defined. While our current understanding suggests that degranulating neutrophils, derived from the aging inflammatory microenvironment, may provide a significant contribution of MMPs to salivary secretions, identifying the cellular origin of MMPs and TIMPs in the aging SG is critical for understanding the mechanism of aging-related degeneration and warrants further investigation.[28]

In addition to observing that fibrosis is strongly associated with aging-related remodeling of SG morphology, a recent study by Li et al. identified several phenotypic characteristics, associated with aging human labial salivary glands (LSGs), including atrophic acini, an increase in inflammatory cells, altered immune response, and intracellular accumulation of lysosomes and autophagosomes.[29] The study used the rat as a natural model of aging to demonstrate that SGs from aged rats have increased amounts of inflammation compared to young rats. Co-localization studies, using fluorescently labelled antibodies, revealed an increase in the distribution of MMP-9+/CD45+ immune cells in aged, but not young, rat sublingual glands. While MMP-9 plays a key catabolic role in tissue remodeling, it is enriched in cells expressing a senescence-associated secretory phenotype (SASP). Further research is

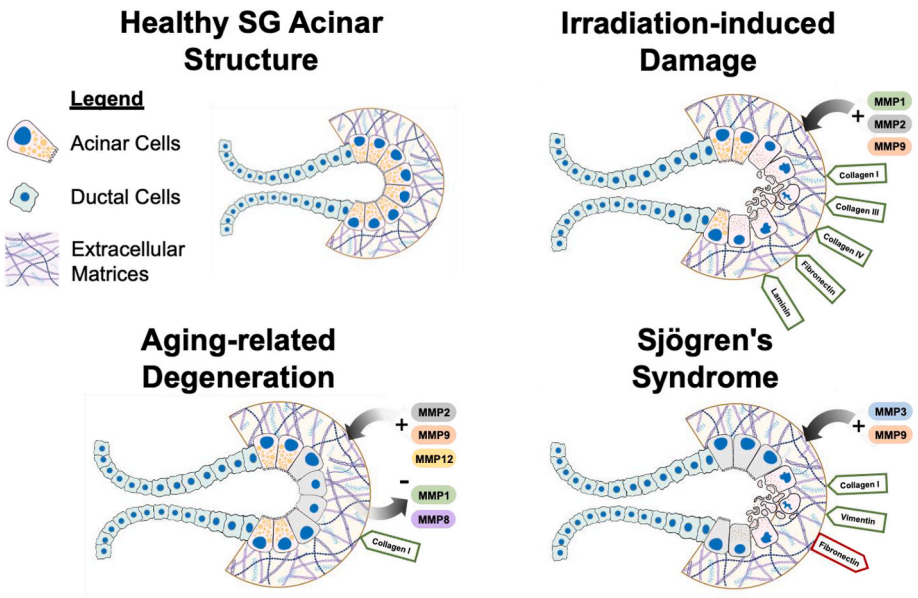

Fig. 1. SG pathologies are characterized by unique changes in the local microenvironment. Different mechanisms of SG damage perturb the expression of MMPs in addition to up/down regulation of specific ECM components. Created using BioRender.com.

needed to better understand the role of MMPs in SG during aging and determine whether these enzymes have potential as therapeutic targets to ameliorate or even reverse SG aging.

2.2. Irradiation-induced SG damage

When the head and neck region is exposed to irradiation (IR), MMP activity in the damaged SG tissue is increased, a process likely related to an increase in inflammation and edema in the damaged gland.[4,30] By use of a *Drosophila* model, Lee et al. showed that IR stimulated programmed cell death in the SG; in addition, there was a significant up-regulation of *MMP-1* expression, coupled with a >20-fold down-regulation of *TIMP*, suggesting that conditions were conducive for high levels of active proteolytic enzyme activity and matrix breakdown.[31]

In a study of IR-induced SG hypofunction in murine SMGs, RNA-seq analysis 300 days post-IR showed an increase in matrix metalloproteinases (*MMP2*, *MMP3*) and a cysteine peptidase inhibitor (*Serping1)* which are involved in ECM remodeling and fibrosis.[32] Moreover, the study also reported that *MMP2* and *Serping1* were similarly upregulated in porcine parotid glands at 16 weeks post-IR. Most recently, Uchida et al. demonstrated that, in bilaterally irradiated murine SMGs, transcriptional- and protein-level expression of MMP2 was significantly increased at 24 and 48 h post-IR.[33]

2.3. Sjögren's syndrome

Sjögren's syndrome (SS) is an autoimmune disorder that primarily affects exocrine glands, including SGs, and leads to a reduction in saliva production. Currently, the etiopathogenesis of SS remains unclear, but lymphocytic infiltration and autoimmune attack have been shown to contribute to acute SG inflammation and ultimately destruction of glandular tissue.[34–36] Although SS represents a distinct pathology, degeneration of the SG microenvironment and dysregulation of MMP activity are consistent features of the disease.[4,18] This assertion is supported by clinical biopsy studies showing extensive amounts of SG ECM remodeling in SS patients,[37] coupled with increased expression of MMP-3 [38] and MMP-9 expression and activity.[39,40] In a clinical study by Pérez et al. SS patients were stratified into three categories, based on disease severity, and MMP and TIMP expression at both the mRNA and protein levels determined.[41] The investigators observed that the severity of lymphocytic infiltration in labial SGs was correlated with pronounced alterations in acinar morphology and reduced acinar cell polarity (i.e., reflected by loss of basal positioning of the nucleus). In addition, the degree of SG damage in these subjects was strongly correlated with an increase in MMP-9/TIMP-1 and MMP-3/TIMP-1 ratios, suggesting that an imbalance in the ratio of MMPs to TIMPs was linked to acinar damage and SS progression.

As stated above, SG cells are known to produce both MMPs and TIMPs. As a result, it's possible that metalloproteinases, which are normally involved in ECM maintenance and remodeling, may play a role in degrading the matrix that contributes to SS pathogenesis. One way that these endogenous enzymes may be activated is through anoikis, which is a programmed cell death process induced by an interruption in integrin-mediated attachment to the ECM, that leads to the deterioration of acini in SS.[38,42] This process also alters the ratio of MMP-9 and MMP-3 to TIMP1 produced by the acinar and ductal cells.[41] Thus, the endogenous SG cells participate in breaking down the secretory machinery and supporting epithelium, even though there is no direct participation by the infiltrating lymphocytes.

A disorganized SG epithelium and basal lamina are hallmarks of SS pathology. Observations made from both animal models and clinical samples suggest that this deterioration in tissue structure and morphology is due to an interplay between inflammation, innate immunity, and matrix metabolism.[43–45] Non-obese diabetic (NOD) mice have been employed as a powerful model for studying autoimmune exocrinopathy because they spontaneously develop SS-like secretory dysfunction by 12–16 weeks of age.[46] Investigations of SG morphology in these mice have described the presence of significant changes in gland structure and homeostasis, along with reduced secretory output, prior to the onset of inflammation.[47] In Cha et al. decreased acinar cell proliferation was coupled with alterations in the structure of the SG epithelium and basal lamina; these changes are believed to be due to increased MMP-2 and MMP-9 activity during SG development.[48] Significantly, these observations suggest that MMP-mediated degradation of the SG ECM may produce peptides that subsequently stimulate an auto-immune response and drive SS pathogenesis (see below).[49] In a clinical study of 19 patients with primary SS, Pérez et al. concluded that increased SG MMP activity correlated with increasing disease severity. Moreover, through immunolocalization studies of SG biopsies, staining intensity for MMP-3 and MMP-9 in acinar and ductal cells was found to be

unrelated to their proximity to inflammation-inducing mononuclear cells.[40] These observations support the hypothesis that lymphocytes, which are routinely found infiltrating the gland in SS, may not be directly responsible for ECM degradation due to dysregulated MMP activity.

An alternative mechanism by which MMP-mediated degradation of the SG basal lamina can stimulate immunopathogenesis is through the generation of ECM breakdown by-products, known as damage-associated molecular patterns (DAMPs).[50] These matrix fragments have been shown to activate sterile inflammation by binding both pattern recognition receptors (PRRs), such as toll-like receptors (i.e. TLR2, TLR4) and other classes of surface receptors (e.g. integrins).[51] PRRs are widely-expressed by cells of the innate immune system (i.e. macrophages, dendritic cells, neutrophils, and mast cells), stromal cells (i.e. epithelial cells and fibroblasts) and endothelial cells. Binding to PRRs stimulates the inflammasome of the innate immune response, MAP kinase, NF-kB signaling pathways, and complement activation cascades which promote lymphocyte recruitment, coupled with the generation of autoantibodies and stimulation of pro-inflammatory cytokines.[50] DAMPs are normally not accessible by the immune system, but they can be released by living or necrotic cells under stress conditions.[52] Clinical studies have shown that MMPs produced by cells from SS patients degrade the SG basal lamina with different degrees of efficiency and that laminin was the most affected followed by fibronectin and collagen types I, III, and IV.[38]

Recent studies have identified SG-specific DAMPs associated with the pathogenesis of SS.[50] An investigation conducted in NOD mice showed that the production of biglycan (Bgn) and decorin (Dcn) was similar in SGs of both healthy and SS mice.[53] However, sera from SS mice contained elevated levels of *anti*-Bgn and -Dcn autoantibodies relative to healthy controls. In addition, the study also found that breakdown of the SG basal lamina in SS mice generated DAMPs associated with these small proteoglycans. SG-associated DAMPs are thought to activate PRRs (TLR2, TLR4) and trigger Myd88-dependent activation of NF-κB, ultimately leading to persistent B cell activation in the SG and contributing to autoimmunity. These observations were further supported by another report showing significantly elevated *DCN* gene expression in SGs of primary SS patients.[54] Immunostaining of SG biopsies indicated co-localization of Dcn and TLR4, a PRR found in the SG epithelium, and staining intensity was found to increase with SS severity. In addition, soluble Dcn was shown to induce the polarization of macrophages from the naïve (M0) to activated (M1) phenotype. Taken together, these studies outline a framework for how matrix proteolysis in SGs, enabled at least in part by dysregulated MMP-activity, may potentiate a runaway feedback loop of autoimmune activation which drives SS etiopathology.

3. Fibrosis is the outcome of different types of SG degeneration and disease

All degenerative processes that dismantle a healthy SG epithelium and basal lamina ultimately lead to fibrosis which is characterized by scar/repair tissue formed in response to MMP-mediated ECM degradation.[55] The overall composition of the fibrotic ECM in SG is known to be distinct from that of healthy tissue.[23,24] In healthy SG, the ECM is mainly composed of proteins and complex carbohydrates (e.g. collagens, lamanins, fibronectin, glycosaminoglycans and proteoglycans).[5] In contrast, fibrotic SG ECM contains increased amounts of certain collagens, lamanins and fibronectin[56] and the distribution and organization of the ECM components are altered (vs. normal healthy tissue).[57,58] In addition to these compositional changes, which lead to the degeneration of acinar and ductal architecture and secretory function, fibrosis also impairs the innervation and microvasculature of the SG, resulting in reduced blood flow and oxygenation and further exacerbating damage to the tissue and its microenvironment.[59,60] Fibrosis of major SGs is not the only cause of fibrosis-induced hyposalivation. Oral submucosal fibrosis also impacts minor salivary glands, which can impair the function of secretory cells in the gland and reduce salivary secretion.[61] Taken together, these changes in tissue organization and regulatory cues in the extracellular environment lead to a significant impairment in gland homeostasis and secretory function.[62,63] Fig. 2 presents a generalized framework showing how different pathways of SG microenvironmental damage all converge on fibrotic remodeling of the ECM.

3.1. Aging-related degeneration

The morphological changes that occur in human SGs during physiological aging have been established by studies showing that acinar volume in major SGs decreases by about 25–30% and fibrosis and adipose infiltration into the glandular tissue is significantly increased.[22] Rodent models have been used to extend these observations and show that both fibrosis and collagen deposition are increased in all three major SGs during aging.[23,64] Moreover, impaired SG function was correlated with an increase in periductal collagen accumulation in acinar and ductal cells. Although the relationship between acinar atrophy and fibrosis in the SG during aging has been relatively well-described, the precise mechanisms responsible for the morphological and functional changes have not been completely defined.

Fibrosis may reduce SG function through a variety of mechanisms. By use of a rodent model of SG aging, Choi et al. suggested that periductal stiffening due to increased collagen deposition may impair SG secretory function and mucin production.[65] Yamauchi et al. also employed a rodent SMG model to show that localized increases in oxidative stress may contribute to many aspects of the aging SG phenotype, including the accumulation of fibrotic tissue.[66] Finally, Tomiskai et al. attributed increased fibrosis and adipose infiltration, histologically observed in a gerbil aging SG model, to the increased number of fibroblasts expressing intracellular type I procollagen and serpin collagen chaperones.[67] They proposed that this increase in collagen secretion by the SG cells was a major driver of aging-related fibrosis and associated salivary hypofunction. Indeed, both alterations in SG cellular populations and microenvironmental cues are likely participants in the progression of aging-related fibrosis.

Observations in aging animal models of SG fibrosis have also been translated to studies of human clinical samples. Leehan et al. investigated the relationship between fibrosis and adipose infiltration in labial SG biopsies from SS and non-SS sicca patients and concluded that there were no significant differences in displacement of SG volume by fatty tissue.[62] The study was able to demonstrate that the relationship between salivary flow and fatty replacement, as well as morphologically identified fibrotic changes, was almost exclusively influenced by patient age and was independent of SS-status. This finding was supported by a 10-year retrospective analysis of minor SG biopsies by Klein et al. which found that natural SG aging (i.e. fibrosis and adipose replacement) contributed to impaired salivary/secretory function, even in the absence of specific pathological processes in SS or IR-induced damage.[63] Overall, most research points to the accumulation of fibrotic tissue in the SG and the resulting impairment in secretory function as a natural process in oral aging physiology. However, fibrosis is also strongly involved in SG pathologies which mainly manifest in the aged. One possibility is that these conditions (e.g. IR damage, SS) may further promote an already on-going fibrogenic process occurring in the aging SG.

3.2. Irradiation-induced SG damage

Generally, IR-induced SG damage is marked by acinar cell atrophy and replacement of the cells by fibrotic tissue.[68] An initial decline in SG function occurs in the first three days after exposure to IR in mice and rats.[4] Mice experience a significant reduction in acinar cells, salivary flow rate, and changes in saliva composition shortly after IR exposure.[69–71] Reports have noted that chronic hyposalivation begins between 30 and 300 days following IR, with fibrosis occurring as early as

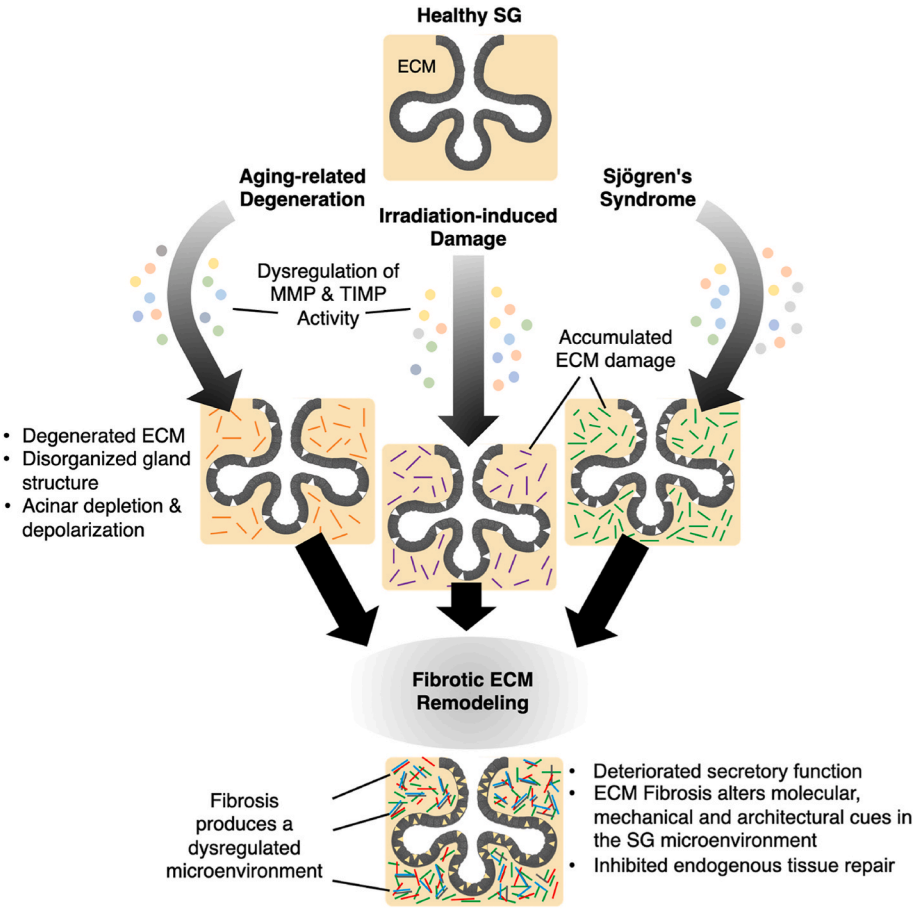

Fig. 2. Fibrosis is the final common pathway of SG damage. Aging-related degeneration, IR-induced damage, and Sjögren's Syndrome are distinguished by different pathways (e.g. expression and activity of MMPs/TIMPs) which degrade the SG microenvironment and contribute to reduced salivary functionality. Irrespective of the pathway involved, fibrotic remodeling emerges in all three types of SG damage as a consequence of improper repair of the damaged ECM. The impact of fibrosis is a permanently compromised SG structure and loss of function. Created using BioRender.com.

30 days after receiving fractionated IR in minipigs and over 4 months in rodents.[72,73] In addition to immunostaining of SG-gland biopsies showing fibrotic lesions, genomic profiling has linked increased collagen deposition in irradiated SGs to intraglandular up-regulation of fibrosis pathways.[32,58] In addition, studies have shown that IR-induced SG fibrosis occurs in defined stages over time that are dependent on radiation dose.[57,74] In a rat SG irradiation model, Friedrich et al. measured the accumulation of type I collagen as a function of radiation dose and concluded that radiation doses exceeding 20 Gy (but less than 60 Gy) promoted periductal and perivascular fibrosis of SMGs.[74] Furthermore, multivariate analysis indicated a statistically significant increase in type I collagen accumulation in the excretory and striated ducts, capsule and septae, nerves and the vascular adventitia of irradiated glands. A subsequent investigation compared the distribution patterns of ECM components in naïve versus irradiated rodent SMGs at 6 and 12 months post-IR and concluded that radiation induces dose-dependent ECM remodeling, with higher doses resulting in an increased amount of fibrosis and a decreased number of acini.[57] In animals not exposed to radiation, the ECM was uniformly distributed throughout the gland, including regions containing acinar cells, ducts, nerve tissues, and vessel walls. However, in irradiated glands, significantly increased staining intensity for ECM proteins was localized within the radiation field, compared to tissue outside the radiation field or in non-irradiated animals. Moreover, the greatest deposition of laminin, fibronectin and type III collagen was most evident in animals irradiated at 60 Gy. The expression of laminin in irradiated glands increased in a dose-dependent manner, particularly in regions previously occupied by acinar cells. Type III collagen staining was also significantly increased in irradiated vs. non-irradiated glands. Fibronectin staining was increased around acinar cells, granular convoluted tubules, and striated and excretory ducts. Finally, type IV collagen staining followed a pattern similar to that of laminin, showing an increase in intensity in irradiated glands, but did not show any significant increase in accumulation at the 20 Gy dose relative to controls.

Fibrosis has been shown to significantly impair SG secretory function by reducing the innervation and drainage of the acinar and ductal structures.[56,75] Finally, a study comparing structural and compositional changes occurring in human SMG at 6 months versus 6 years post-IR therapy, showed that collagen density in the fibrotic lesions increased significantly over time and was accompanied by a gradual clearing of ECM-forming adipocytes as the collagenous fibers in the matrix became more tightly organized.[58]

Although the formation of fibrotic lesions after IR damage has been conclusively demonstrated to occur in both animal models and human subject biopsies, the mechanisms which mediate this process in SG remain to be elucidated.[76,77] One potential signal transduction pathway involved in the SG damage response to IR is TGF-β, a master regulatory cytokine for fibrosis.[55] TGF-β binds to type I & II transmembrane serine/threonine kinase receptors to activate downstream signaling cascades of SMADs, which ultimately stimulate ECM remodeling and promote fibrosis by regulating various transcriptional targets.[46,78] In human patients, the expression of TGF-β has been shown to be elevated following radiotherapy of the head and neck,[79] as well as in murine models of IR-induced salivary hypofunction.[80] TGF-β promotes fibrosis by increasing ECM synthesis, activating fibroblasts, inducing acquisition of the myofibroblast phenotype, and stimulating the epithelial–mesenchymal transition (EMT) and endothelial-mesenchymal transition (EndMT).[81] Additional research is necessary to conclusively delineate the mechanisms involved in the SG response to IR, including the prospective role of TGF-β as a mediator of fibrosis.

3.3. Sjögren's syndrome

The histological hallmarks of SS in SG biopsies are the presence of a lymphocytic infiltrate and loss of healthy exocrine tissue architecture, including acinar atrophy, destruction of the basal lamina and epithelium, hyperplasia of the intraglandular duct lining, and interstitial fibrosis.[82–85] Typically, fibrosis in the SG manifests as regions of ECM hyperplasia, where healthy glandular architecture is disrupted by the deposition of a fibrous dense type I collagen network.[86,87] While fibrosis may be more pronounced in the advanced stages of SS, studies have shown that the extent of fibrosis correlates with the degree of lymphocytic infiltration and severity of SS symptoms (e.g., inflammation scores; stimulated salivary flow rate).[88,89] The identification of age-related fibrosis in healthy individuals has caused much uncertainty regarding the use of this marker in SS patients as its presence may actually be a consequence of aging rather than a manifestation of pathology. Moreover, fibrosis in SS patients is further complicated by its relatively late onset, usually in the fourth decade of life or later. Llamas-Gutierrez et al. sought clarification of this issue by evaluating a series of SG biopsy specimens (i.e. 63 cases of pSS and 11 healthy controls) and found that pSS patients had a higher grade of fibrosis and confirmed that there was a connection between fibrosis and SS after adjusting for age.[9]

Fibrotic remodeling of the SG microenvironment in SS is not limited to increased deposition of type I collagen. Laminins are a family of structural glycoproteins which play a crucial role in organizing the structure of the SG and maintaining its function, including the formation of a network of fibers that support the glandular epithelium which is responsible for producing and secreting saliva.[5] Within the inter-acinar basal lamina of the SG, laminins and type IV collagen form a major structural network which is stabilized through non-covalent crosslinks of globular nidogen proteins.[42] Studies have shown that remodeling of the basal lamina during fibrosis significantly alters laminin organization and the support/integrity of the salivary acini and ductal structures.[90] A comparison of laminins in healthy SGs versus those in SS showed a significant decrease in the abundance of almost all isoforms of laminin, along with a severely disorganized laminin structure in the basal lamina.[91,92] To better understand the changes occurring in the LSG basal lamina of SS patients, Kwon et al. analyzed the levels of laminin α1, α4 and γ2 chains, as well as nidogens 1 and 2, at the mRNA and protein levels. The results showed that patients with low levels of inter-acinar fibrosis had elevated mRNA and protein levels for unprocessed laminin γ2 and less pronounced changes in subjects with high fibrosis. Only protein-level changes were observed for laminin α1 and α4 chains in low fibrosis patients. Nidogens 1 and 2 displayed similar mRNA and protein levels in SS patients and control subjects, but high levels of nidogen degradation products were observed in low fibrosis patients. These results suggest that active remodeling of the basal lamina occurs during the early stages of disease, potentially contributing to the disorganized basal lamina observed in patients with low inter-acinar fibrosis.[42] Work by Konttinen et al. showed that, apart from the general structural anomalies noted for SG acini in SS, the laminin α1 chain is depleted from the acinar basement membrane, reducing the capacity of the local microenvironment to regulate differentiation of progenitors to acinar cells.[93] Taken together, these results indicate that changes in laminin and collagen isoforms in SS contribute to morphological changes in the SG and potentially influence gland function.[91]

Although ECM remodeling during fibrosis plays a critical role in the pathogenesis of SS, specific factors that induce SG fibrosis are yet to be clearly identified. To address this gap in our understanding, Mao et al. used a murine model of SMG fibrosis to determine if increased permeability of the tight junction (TJ)-based endothelial barrier, which is crucial in glandular homeostasis, contributes to SG fibrosis.[94] The results showed that claudin-5 expression was increased in fibrotic glands, while the TJ sealer, AT1001, was able to restore the function of the endothelial barrier and attenuate SG fibrosis. Moreover, the study also confirmed that claudin-5 was upregulated in fibrotic SMGs, along with elevated p-ERK1/2 signaling, from patients with chronic SG inflammation. Overall, the study provided convincing evidence that impaired endothelial barrier function plays a role in the progression of SG fibrosis and provided a mechanistic framework for the pathogenesis of ECM remodeling in inflammatory conditions such as SS.

Similar to IR-induced SG damage, recent studies have suggested a link between chronic inflammation in SS and SG fibrosis via activation of the EMT in the SG epithelium. Sisto et al. investigated the expression of E-cadherin (an epithelial cell marker) and the mesenchymal markers, vimentin and type I collagen in SG biopsies from SS patients.[87] The results indicated that increased SG tissue inflammation in SS was negatively correlated with E-cadherin expression and positively correlated with the expression of vimentin and type I collagen. In addition, the study showed that IL-17 and IL-22 synergistically promoted the EMT in human SG epithelial cells (SGECs) from healthy subjects, including the upregulation of mesenchymal markers and suppression of epithelial markers. The results suggest that inflammatory mediators, including IL-17 and IL-22, may play an important role in the initiation and progression of fibrosis in primary SS, and as such, constitute potential therapeutic targets for reducing fibrosis and protecting SG function.

4. Fibrosis of the SG microenvironment is an obstacle to effective regenerative therapies

Generally, regenerative medicine-based therapies for SG hypofunction have not been very successful because, in our opinion, aging, disease or physical insult have produced major changes to the SG microenvironment that result in loss of important regulatory cues. These changes lead to SG hypofunction via self-propagating, mutually-reinforcing, multifactorial mechanisms that result in secretory failure.[95] The cumulative effect of these aberrant regulatory signals to SG regeneration are summarized in Fig. 3. For example, at least three types of cell damage account for the decrease in saliva secretion observed in irradiated SGs: cell membrane damage caused by free radicals, changes in the signaling pathways that regulate the production of saliva (such as Ca^{2+} signaling), and inactivation of ion channels and transporters (e.g. aquaporins).[96] Thus, attempts to increase the expression of aquaporins (e.g. aquaporin-1 [*AQP1*]) have demonstrated a degree of clinical effectiveness, but the benefits to salivation are both limited in impact and temporary.[97,98] This is likely because improvements in secretory function provided by the gene therapy are counteracted by the loss or damage to regulatory cues in the microenvironment.[99] Other genes, such as nerve growth factor and neurturin, have shown promise in alleviating radiation-induced SG dysfunction in animal models.[32] However, their efficacy and duration are also influenced by the dysregulated cues in the microenvironment.[100] While gene therapies may temporarily reduce symptoms, their combination with strategies to restore the damaged microenvironment may offer a more effective long-lasting cure for SG hypofunction.

Similarly, potential new therapies focused on restoring SG cells and tissues using stem cells or SG progenitors must also contend with dysregulated cues in the damaged SG microenvironment.[101] Depending on the degree of SG damage, the regenerative or anti-inflammatory potential of the implanted cells may not be sufficient to overcome the deleterious influence of the damaged microenvironment or hijack the fibrogenic potential of stem cells (or stromal cells) to increase ECM remodeling and fibrosis (Fig. 3). Senolytic approaches, which aim to selectively eliminate damaged or senescent SG progenitors, may be a promising approach for promoting endogenous regeneration in the damaged SG.[102] However, the effectiveness of this intervention is also subject to the accumulation of deleterious cues in the SG tissue and their effect on the cells.[103,104]

At present, we only have limited knowledge regarding the capacity of implanted cells to repair damaged ECM or fibrotic tissue.[105,106] *In vitro* cell culture models have only recently been developed to assess changes in mesenchymal stem cell (MSC) behavior when maintained in

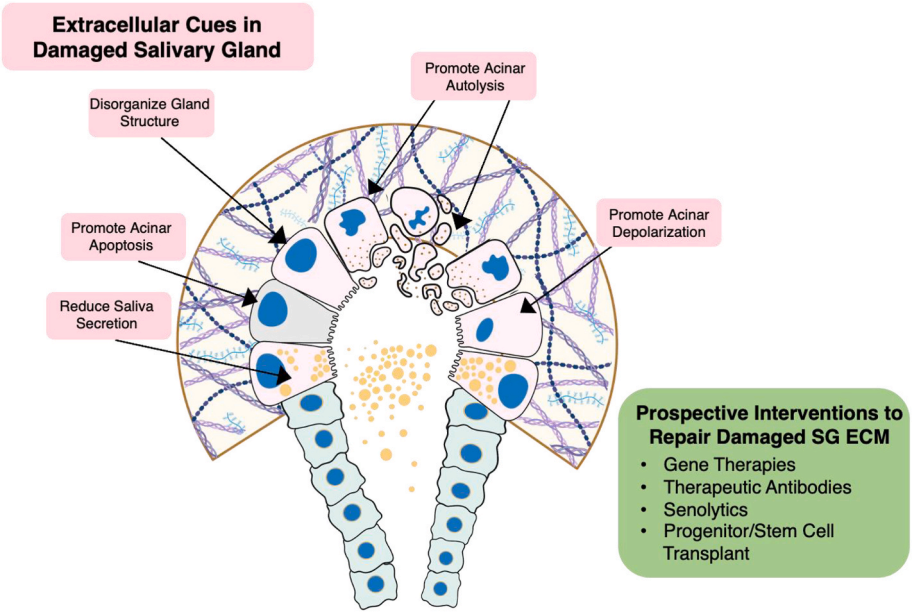

Fig. 3. Damaged extracellular cues in SG disease. The damaged SG microenvironment produces aberrant cues which contribute to dysregulated of SG organization, cell behavior and function. If unaddressed, these degenerative cues interfere with SG regeneration. Created using BioRender.com.

"healthy" versus "diseased"/"damaged" microenvironments. Our group conducted a study using decellularized ECMs produced by bone marrow stromal cells (MSCs) obtained from "young" and "elderly" donors as novel *in vitro* cell culture surfaces. We found that when young MSCs were cultured on the ECM derived from elderly MSCs, they exhibited significant, deleterious changes in their phenotype, including reduced sensitivity to osteogenic growth factors such as BMP-2 and IGF-1.[107] Burk et al. used a similar approach to create MSC-derived ECMs produced under regular versus pro-fibrotic conditions (e.g. TGF β1)[108] and observed substantial differences in the collagen content and architecture (i.e., tissue culture plate and Matrigel-ECM versus TGFβ1-ECM). When MSCs were cultured on these matrices, they showed significant differences in the expression of genes related to matrix metabolism. Notably, MSCs cultured on the pro-fibrotic ECM up-regulated MMP expression while down-regulating the expression of key MMP inhibitors, TIMP-1 and TIMP-2, suggesting that the fibrotic microenvironment modulates matrix synthesis by enhancing degradation in cultures. The study suggested that a damaged microenvironment can induce healthy MSCs to express a myofibroblast-like, pro-fibrotic phenotype.

While these *in vitro* studies do not directly investigate aging-related changes in the SG microenvironment, the results can be extended to the use of stem cells for restoring SG function by determining if a damaged microenvironment can overcome the regenerative potential of multipotent stem cells. Numerous studies, referenced above, have shown that physiological aging, IR-induced injury, and SS substantially damage the SG microenvironment by changing the architecture and composition of the local ECM through both altered matrix metabolism (i.e. MMPs) and fibrotic remodeling. Under normal conditions, these extracellular regulatory cues maintain tissue homeostasis. When perturbed, the aberrant cues reduce the functional capacity of the gland and contribute significantly to pathologies associated with all three modes of SG damage. In order to be effective in reversing this damage, both cell and gene therapies must overcome powerful cues in the extracellular regulatory environment which favor SG dysfunction.

5. Restoration of the ECM should be the first target of stem cell-based SG regeneration

Restoration or repair of damage to the SG ECM caused by aging, disease or physical insult and mediated by MMPs and fibrosis, may enable new stem cell-based and gene therapies to repair or regenerate tissue and perhaps even reverse the loss of secretory function. Hyper-expression of MMP-9 in the salivary epithelium of patients with pSS causes severe ECM remodeling and characteristic degeneration of glandular tissue.[38,40,109] By employing primary SG epithelial cells derived from pSS patients to study the regulation of MMP-9 expression, Noll et al. identified overexpression of a key transcription factor, ETS1.[110] siRNA knockdown of ETS1 reduced MMP-9 expression at both transcriptional and protein levels and represents a potential therapeutic target for inhibiting MMP-mediated degradation of the SG microenvironment in aging and disease.

While *in situ* protection or repair of damaged SG ECM would be an ideal goal of regenerative therapies, current tissue engineering methods, which address the degenerated ECM, generally employ synthetic scaffolds combined with stem cells or progenitors. Other approaches have employed molecular or genetic methods to directly modulate the composition of the SG ECM. For example, significant changes in the shape and function of acinar and ductal cells in labial SGEC cultures treated with *anti*-SSA (Sjögren's-syndrome-related antigen A) autoantibodies were accompanied by alterations in the expression of fibulins, a group of ECM proteins which regulate cell shape and function.[111] These results suggested that *anti*-SSA autoantibodies modulate the expression of ECM proteins by SG epithelial cells and contribute to SS pathogenesis. Subsequently, Lisi et al. investigated the possibility of using antibodies to regulate the expression of fibulins by SGECs as a potential approach for mitigating damage to SG architecture in SS.[37] The results showed that *anti*-SSA antibodies were quite effective at reducing the expression of fibulin-6 which plays a role in disorganizing the SG ECM and the loss of glandular structure and function seen in SS.

Other approaches which have attempted to modulate damage to the SG microenvironment have focused on reducing fibrosis. In a murine IR-induced damage model, Lombaert et al. employed an adeno-associated virus serotype 2 (AAV2) vector to carry the human neurotrophic factor, neurturin (CERE-120), to treat injured SGs.[32] The results showed that CERE-120 had the potential to alleviate salivary hypofunction by diminishing periductal and perivascular fibrosis. Genomic analysis of SGs treated with the CERE-120 vector displayed reduced expression of genes associated with fibrotic ECM remodeling, *MMP2*, *MMP3* and *Serping1*. Another study used a transgenic knockout (KO) mouse model to demonstrate that deletion of differentiated embryo chondrocyte

expressed gene-1 (Dec1), a transcription factor and major regulator of cellular senescence, has the potential to alleviate age-related decline in SMG function.[25] Significantly, the study showed that loss of transcription factor expression simultaneously reduced the loss of SMG function (due to fibrosis) and expression of type I collagen and MMP-2 during aging in Dec1-KO mice. These results suggest that *Dec1* siRNA has potential as a prospective therapy for protecting SG integrity and function during aging. Others have proposed the administration of chimeric antigen receptor T-cells (CAR-T) to selectively target myofibroblasts expressing fibroblast activation protein (FAP).[112] While these approaches demonstrate the therapeutic potential of moderating fibrosis to preempt degeneration of the SG microenvironment, an approach which repairs the SG ECM *in situ* has yet to be identified.

MSCs have the potential to slow, moderate or even repair SG damage *in vivo* as a result of aging, IR, or SS, through multiple pathways.[113] Unfortunately, most studies of MSC-mediated SG regeneration or repair have emphasized the ability of the MSCs to serve as a reservoir of progenitor cells without addressing their potential to mediate SG matrix repair. However, the capacity of MSCs to moderate inflammation through cell-cell contact, paracrine factor secretion, and production of cytokine-laden extracellular vesicles (EVs) may also play an important role in modulating the degradation/repair of the SG microenvironment.[114] Based on these putative immunomodulatory and anti-inflammatory capabilities, we speculate that the administration of MSCs at relatively early stages of ECM damage may reduce deterioration and fibrosis of the native SG microenvironment, which if left unchecked can severely impair the function of SG cells and create major obstacles to endogenous tissue repair by resident cells. Cell culture studies have shown that cell-cell contact between fibroblasts and MSCs and paracrine factors secreted by MSCs have a potent regulatory effect on the inflammatory response of fibroblasts, reducing their expression of inflammation-associated adhesion molecules (e.g., ICAM1 and VCAM1) and attenuating the expression and activation of ECM-degrading MMPs.[115–117] Other studies have suggested that MSC conditioned media have the ability to both reduce inflammation and ECM deposition in highly damaged, irradiated human cardiac fibroblasts[118] and human keloid fibroblasts[119] via inhibition of Notch and Smad signaling and the expression of TGF-β2.[120] While these studies were not performed in SG-derived cell types, they suggest that MSCs have the potential to reduce ECM degradation and fibrosis, which may be leveraged to mitigate SG damage.

Recently, *in vivo* models of liver fibrosis and cardiac ischemia have been used to investigate the anti-fibrotic potential of both MSCs and their paracrine products. In a novel zebrafish embryo model of induced liver fibrosis, van der Helm et al. showed that the injection of bone marrow-derived MSCs into embryos with liver fibrosis-inducing agents, significantly reduced the expression of collagen and TGF-β as compared to untreated controls.[121] Kore et al. employed an acute murine cardiac ischemia model to investigate the potential of MSC-derived exosomes in reducing cardiac diastolic dysfunction by inhibiting the profibrotic-phase of matrix remodeling and/or initiating regenerative remodeling of the damaged tissue.[122] The results showed that MSC exosomes suppressed inflammation and promoted ECM remodeling in hearts with acute and chronic ischemia by reducing the secretion of fibronectin and modulating collagen production. In addition, proteomic analyses in this study showed that IL-1β expression was reduced and profibrotic signaling inhibited in the hearts of animals treated with MSC exosome.

Finally, a major potential advantage of using MSCs to treat SG damage is their potential to not only mitigate fibrosis and its effects, but also to repair the damaged SG microenvironment. To achieve this goal, transplanted stem cells must be able to overcome the destructive microenvironmental cues that have (presumably) activated EMT or apoptotic programs in SG stem cells/progenitors already present in the damaged tissue.[123] Thus, in order to maximize the restorative potential of multipotent MSCs, transplantation of the cells must occur at a relatively early stage of tissue damage, prior to the onset of fibrosis, before the local microenvironment is compromised beyond repair. For example, MSCs can suppress inflammation in early wound healing via TGF-β1, but in later remodeling phases, the same growth factor may actually increase myofibroblast differentiation and promote fibrosis.[124,125] Indeed, other studies have convincingly shown that while MSCs may improve healing during the early stages of fibrosis, when transplanted at more advanced stages, they respond to cues in the microenvironment that exacerbate ongoing fibrosis, rather than resolving or reversing any existing damage.[126,127]

A potential solution that may mitigate MSC-mediated fibrosis involves developing methods which "reeducate" MSCs during *in vitro* clonal expansion, a process necessary for generating the large numbers of cells required by regenerative therapies.[1] Unfortunately, the expansion of MSCs in artificial cell culture environments (e.g., tissue culture plastic) reduces self-renewal and differentiation capacity, as well as stimulating overall phenotypic drift, which diminishes the expression of stem cell markers and anti-inflammatory capacity over multiple passages.[128–130] Importantly, Majd et al.[131] and Talele et al.[132] outlined a mechanistic pathway linking culture on high-stiffness substrates such as TCP to MSC differentiation to a pro-fibrotic, myofibroblast phenotype, via upregulation of α-smooth muscle actin stress fibers and subsequent nuclear translocation of YAP/TAZ.

To address the susceptibility of stem cells to culture environments which alter their phenotype and promote fibrosis, our group and others have demonstrated that MSCs from various tissues (e.g. bone marrow, adipose and cardiac tissues) may be maintained on stromal cell-derived ECMs which recapitulate some of the biochemical (e.g., proteins, proteoglycans), mechanical, and architectural attributes of tissue-specific MSC niches.[7,107,133,134] Culture environments which reproduce these native properties are better able to preserve self-renewal capacity and stem cell phenotype during expansion[135–138] and restrain the therapeutically undesirable transition to pro-fibrotic phenotypes.[131,132] In this regard, Li et al. provided evidence that "soft priming" MSCs during expansion reeducated their mechanical memory and suppressed MSC fibrogenic response. The study showed that even when subsequently transplanted into scar-like wound environments, MSCs maintained a mechanosensory memory, including expression of myocardin-related transcription factor-A (MRTF-A).[139] Our group recently demonstrated that MSCs, pre-incubated with SG-ECM can induce MSC *trans*--differentiation to SG lineage cells both *in vitro* and in an *in vivo* rodent model.[113] Taken together, these recent studies suggest that while the sensitivity of MSCs to signals in the microenvironment is central to their regenerative potential, careful consideration must be given to the cues to which they are exposed while being prepared for study and therapeutic use. Moreover, if MSCs are exposed to appropriate cues during expansion, they may retain their ability to restore a compromised microenvironment, including the replacement of a fibrotic matrix which occurs in SG damage.

6. Conclusion

Catabolism (MMPs) and anabolism (deposition of a fibrotic matrix) are opposing processes which are responsible for producing a SG ECM which impairs (or interrupts) both gland homeostasis and secretory function. Fibrosis of the SG is known to occur as a result of aging and as a consequence of multiple pathological processes such as IR-induced injury or autoimmune attack in SS. SG fibrosis represents a formidable obstacle to all forms of potential regenerative therapies. Reversing the ECM damage caused by MMP activity and fibrosis to restore the structural integrity of the glandular tissue and create a more supportive microenvironment for the survival, proliferation, and differentiation of stem cells used in cell-based approaches represents a major challenge. Similarly, reversing the changes to the SG microenvironment caused by aging will improve the efficacy of gene therapies that aim to introduce functional genes into the damaged glandular tissue. We speculate that a

combination of MSC-based approaches for restoring the SG microenvironment with prospective molecular and gene therapies offers a new SG regenerative paradigm because it offers the potential to comprehensively address both cellular and microenvironmental factors which contribute to impaired SG function.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Chen is a Board member and shareholder in StemBioSys, Inc. (San Antonio, TX). Dr. Marinkovic is a shareholder and member of the Scientific Advisory Board of StemBioSys, Inc. (San Antonio, TX). All other authors have no financial or competing interests to declare.

Acknowledgments

This review was supported by NIDCR/NIH R01DE025286 (Yeh & Chen), R21 DE028271A (Yeh & Salmon), VA Merit Review 1I01BX002145-01 (Chen), VA Career Development Award 1IK2BX005694-01 (Marinkovic), as well as pilot funding from the San Antonio Nathan Shock (5P30AG013319-29) and Claude D. Pepper Centers (P30AG044271).

References

1 Marinkovic M, Dean DD, Chen XD. Maintenance and culture of MSCs. In: Chen X-D, ed. *A Roadmap to Non-hematopoietic Stem Cell-Based Therapeutics*. Academic Press; 2019:39–61. https://doi.org/10.1016/b978-0-12-811920-4.00003-3. Published online 2019.
2 Karamanos NK, Theocharis AD, Piperigkou Z, et al. A guide to the composition and functions of the extracellular matrix. *FEBS J*. 2021;288(24):6850–6912. https://doi.org/10.1111/febs.15776.
3 Muncie JM, Weaver VM. The physical and biochemical properties of the extracellular matrix regulate cell fate. *Curr Top Dev Biol*. 2018;130:1–37. https://doi.org/10.1016/bs.ctdb.2018.02.002.
4 Jasmer KJ, Gilman KE, Forti KM, Weisman GA, Limesand KH. Radiation-induced salivary gland dysfunction: mechanisms, therapeutics and future directions. *J Clin Medicine*. 2020;9(12):4095. https://doi.org/10.3390/jcm9124095.
5 Sequeira SJ, Larsen M, DeVine T. Extracellular matrix and growth factors in salivary gland development. *Front Oral Biol*. 2010;14:48–77. https://doi.org/10.1159/000313707.
6 Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. *Nat Rev Mol Cell Biol*. 2014;15(12):802–812. https://doi.org/10.1038/nrm3896.
7 Marinkovic M, Dai Q, Gonzalez AO, et al. Matrix-bound Cyr61/CCN1 is required to retain the properties of the bone marrow mesenchymal stem cell niche but is depleted with aging. *Matrix Biol*. 2022;111:108–132. https://doi.org/10.1016/j.matbio.2022.06.004.
8 Levi N, Papismadov N, Solomonov I, Sagi I, Krizhanovsky V. The ECM path of senescence in aging: components and modifiers. *FEBS J*. 2020;287(13):2636–2646. https://doi.org/10.1111/febs.15282.
9 Llamas-Gutierrez FJ, Reyes E, Martínez B, Hernández-Molina G. Histopathological environment besides the focus score in Sjögren's syndrome. *Int J Rheum Dis*. 2014;17(8):898–903. https://doi.org/10.1111/1756-185x.12502.
10 Marcott S, Dewan K, Kwan M, Baik F, Lee YJ, Sirjani D. Where dysphagia begins: polypharmacy and xerostomia. *Fed Pract Heal Care Prof Va Dod Phs*. 2020;37(5):234–241.
11 Liu F, Wang S. Molecular cues for development and regeneration of salivary glands. *Histol Histopathol*. 2013;29(3):305–312. https://doi.org/10.14670/hh-29.305.
12 Chibly AM, Aure MH, Patel VN, Hoffman MP. Salivary gland function, development, and regeneration. *Physiol Rev*. 2022;102(3):1495–1552. https://doi.org/10.1152/physrev.00015.2021.
13 Tucker AS. Salivary gland development. *Semin Cell Dev Biol*. 2007;18(2):237–244. https://doi.org/10.1016/j.semcdb.2007.01.006.
14 Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. *Adv Clin Exp Medicine Official Organ Wroclaw Medical Univ*. 2014;25(2):383–390. https://doi.org/10.17219/acem/30428.
15 Patel VN, Hoffman MP. Salivary gland development: a template for regeneration. *Semin Cell Dev Biol*. 2014;25:52–60. https://doi.org/10.1016/j.semcdb.2013.12.001.
16 Ogbureke KUE, Fisher LW. Expression of SIBLINGs and their partner MMPs in salivary glands. *J Dent Res*. 2004;83(9):664–670. https://doi.org/10.1177/154405910408300902.
17 Nagel H, Laskawi R, Wahlers A, Hemmerlein B. Expression of matrix metalloproteinases MMP-2, MMP-9 and their tissue inhibitors TIMP-1, -2, and -3 in benign and malignant tumours of the salivary gland. *Histopathology*. 2004;44(3):222–231. https://doi.org/10.1111/j.0309-0167.2004.01814.x.
18 Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol*. 2014;15(12):786–801. https://doi.org/10.1038/nrm3904.
19 D'Amico F, Skarmoutsou E, Mangano K, Malaponte G, Mazzarino MC. Isoproterenol modulates matrix metalloproteinase-2 (MMP-2) and its tissue inhibitor-2 (TIMP-2) in rat parotid gland. *Arch Oral Biol*. 2013;58(4):370–376. https://doi.org/10.1016/j.archoralbio.2012.08.009.
20 Fox RI, Kang HI, Ando D, Abrams J, Pisa E. Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. *J Immunol*. 1994;152(11):5532–5539. https://doi.org/10.4049/jimmunol.152.11.5532.
21 Wu AJ, Lafrenie RM, Park C, et al. Modulation of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) by interferon-γ in a human salivary gland cell line. *J Cell Physiol*. 1997;171(2):117–124. https://doi.org/10.1002/(sici)1097-4652(199705)171:2<117::aid-jcp1>3.0.co;2-r.
22 Toan NK, Ahn SG. Aging-related metabolic dysfunction in the salivary gland: a review of the literature. *Int J Mol Sci*. 2021;22(11):5835. https://doi.org/10.3390/ijms22115835.
23 Tumer MK, Cicek M. Differential immunohistochemical expression of type I collagen and matrix metalloproteinase 2 among major salivary glands of young and geriatric mice. *J Appl Oral Sci*. 2018;26, e20170484. https://doi.org/10.1590/1678-7757-2017-0484.
24 Azevedo-Alanis LR, Tolentino ES, Assis GF, Cestari TM, Lara VS, Damante JH. Acinar autolysis and mucous extravasation in human sublingual glands: a microscopic postmortem study. *J Appl Oral Sci*. 2015;23(5):459–466. https://doi.org/10.1590/1678-775720150139.
25 Yoshida K, Wang X, Bhawal UK. Dec1 deficiency restores the age-related dysfunctions of submandibular glands. *J Physiology Pharmacol Official J Pol Physiological Soc*. 2021;72(4). https://doi.org/10.26402/jpp.2021.4.09.
26 Nassar M, Hiraishi N, Islam MdS, Otsuki M, Tagami J. Age-related changes in salivary biomarkers. *J Dent Sci*. 2014;9(1):85–90. https://doi.org/10.1016/j.jds.2013.11.002.
27 Holmström SB, Lira-Junior R, Zwicker S, et al. MMP-12 and S100s in saliva reflect different aspects of periodontal inflammation. *Cytokine*. 2019;113:155–161. https://doi.org/10.1016/j.cyto.2018.06.036.
28 Uitto V, Suomalainen K, Sorsa T. Salivary collagenase. Origin, characteristics and relationship to periodontal health. *J Periodontal Res*. 1990;25(3):135–142. https://doi.org/10.1111/j.1600-0765.1990.tb01035.x.
29 Li N, Ye Y, Wu Y, et al. Alterations in histology of the aging salivary gland and correlation with the glandular inflammatory microenvironment. *iScience*. 2023;26(5), 106571. https://doi.org/10.1016/j.isci.2023.106571.
30 Walker MP, Wichman B, Cheng AL, Coster J, Williams KB. Impact of radiotherapy dose on dentition breakdown in head and neck cancer patients. *Pract Radiat Oncol*. 2011;1(3):142–148. https://doi.org/10.1016/j.prro.2011.03.003.
31 Lee CY, Clough EA, Yellon P, Teslovich TM, Stephan DA, Baehrecke EH. Genome-wide analyses of steroid- and radiation-triggered programmed cell death in drosophila. *Curr Biol*. 2003;13(4):350–357. https://doi.org/10.1016/s0960-9822(03)00085-x.
32 Lombaert IMA, Patel VN, Jones CE, et al. CERE-120 prevents irradiation-induced hypofunction and restores immune homeostasis in porcine salivary glands. *Mol Ther - Methods Clin Dev*. 2020;18:839–855. https://doi.org/10.1016/j.omtm.2020.07.016.
33 Uchida H, Ingalls MH, Maruyama EO, et al. Short-term and bystander effects of radiation on murine submandibular glands. *Dis Model Mech*. 2022;15(11), dmm049570. https://doi.org/10.1242/dmm.049570.
34 Brito-Zerón P, Baldini C, Bootsma H, et al. Sjögren syndrome. *Nat Rev Dis Prim*. 2016;2(1), 16047. https://doi.org/10.1038/nrdp.2016.47.
35 Xanthou G, Polihronis M, Tzioufas AG, Paikos S, Sideras P, Moutsopoulos HM. "Lymphoid" chemokine messenger RNA expression by epithelial cells in the chronic inflammatory lesion of the salivary glands of Sjögren's syndrome patients: possible participation in lymphoid structure formation. *Arthritis Rheum*. 2001;44(2):408–418. https://doi.org/10.1002/1529-0131(200102)44:2<408::aid-anr60>3.0.co;2-0.
36 Asatsuma M, Ito S, Watanabe M, et al. Increase in the ratio of matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 in saliva from patients with primary Sjögren's syndrome. *Clin Chim Acta*. 2004;345(1-2):99–104. https://doi.org/10.1016/j.cccn.2004.03.006.
37 Lisi S, M D, Scagliusi P, Mitolo V, Sisto M. Anti-Ro/SSA autoantibody-mediated regulation of extracellular matrix fibulins in human epithelial cells of the salivary gland. *Scand J Rheumatol*. 2009;38(3):198–206. https://doi.org/10.1080/03009740802520722.
38 Goicovich E, Molina C, Pérez P, et al. Enhanced degradation of proteins of the basal lamina and stroma by matrix metalloproteinases from the salivary glands of Sjögren's syndrome patients: correlation with reduced structural integrity of acini and ducts. *Arthritis Rheum*. 2003;48(9):2573–2584. https://doi.org/10.1002/art.11178.
39 Konttinen Yrjö T, Halinen S, Hanemaaijer R, et al. Matrix metalloproteinase (MMP)-9 type IV collagenase/gelatinase implicated in the pathogenesis of Sjögren's syndrome. *Matrix Biol*. 1998;17(5):335–347. https://doi.org/10.1016/s0945-053x(98)90086-5.
40 Pérez P, Goicovich E, Alliende C, et al. Differential expression of matrix metalloproteinases in labial salivary glands of patients with primary Sjögren's syndrome: mechanisms of exocrine parenchyma destruction. *Arthritis Rheum*. 2000;43(12):2807–2817. https://doi.org/10.1002/1529-0131(200012)43:12<2807::aid-anr22>3.0.co;2-m.

41 Pérez P, Kwon Y, Alliende C, et al. Increased acinar damage of salivary glands of patients with Sjögren's syndrome is paralleled by simultaneous imbalance of matrix metalloproteinase 3/tissue inhibitor of metalloproteinases 1 and matrix metalloproteinase 9/tissue inhibitor of metalloproteinases 1 ratios. *Arthritis Rheum*. 2005;52(9):2751–2760. https://doi.org/10.1002/art.21265.

42 Kwon Y, Pérez P, Aguilera S, et al. Involvement of specific laminins and nidogens in the active remodeling of the basal lamina of labial salivary glands from patients with Sjögren's syndrome. *Arthritis Rheum*. 2006;54(11):3465–3475. https://doi.org/10.1002/art.22177.

43 Iozzo RV, Schaefer L. Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. *Matrix Biol*. 2015;42. https://doi.org/10.1016/j.matbio.2015.02.003.

44 Sorokin L. The impact of the extracellular matrix on inflammation. *Nat Rev Immunol*. 2010;10(10):712–723. https://doi.org/10.1038/nri2852.

45 Frevert CW, Felgenhauer J, Wygrecka M, Nastase MV, Schaefer L. Danger-associated molecular patterns derived from the extracellular matrix provide temporal control of innate immunity. *J Histochem Cytochem*. 2017;66(4):213–227. https://doi.org/10.1369/0022155417740880.

46 Park YS, Gauna AE, Cha S. Mouse models of primary Sjogren's syndrome. *Curr Pharmaceut Des*. 2015;21(18):2350–2364. https://doi.org/10.2174/1381612821666150316120024.

47 Rosignoli F, Roca V, Meiss R, Leceta J, Gomariz RP, Leirós CP. Defective signalling in salivary glands precedes the autoimmune response in the non-obese diabetic mouse model of sialadenitis. *Clin Exp Immunol*. 2005;142(3):411–418. https://doi.org/10.1111/j.1365-2249.2005.02930.x.

48 Cha S, Blockland SCA, Versnel MA, et al. Abnormal organogenesis in salivary gland development may initiate adult onset of autoimmune exocrinopathy. *Exp Clin Immunogenet*. 2001;18(3):143–160. https://doi.org/10.1159/000049194.

49 Yamachika S, Nanni JM, Nguyen KH, et al. Excessive synthesis of matrix metalloproteinases in exocrine tissues of NOD mouse models for Sjögren's syndrome. *J Rheumatol*. 1998;25(12):2371–2380.

50 Ming B, Zhu Y, Zhong J, Dong L. Immunopathogenesis of Sjogren's syndrome: current state of DAMPs. *Semin Arthritis Rheum*. 2022;56, 152062. https://doi.org/10.1016/j.semarthrit.2022.152062.

51 Denning NL, Aziz M, Gurien SD, Wang P. DAMPs and NETs in sepsis. *Front Immunol*. 2019;10:2536. https://doi.org/10.3389/fimmu.2019.02536.

52 Pandolfi F, Altamura S, Frosali S, Conti P. Key role of DAMP in inflammation, cancer, and tissue repair. *Clin Therapeut*. 2016;38(5):1017–1028. https://doi.org/10.1016/j.clinthera.2016.02.028.

53 Kiripolsky J, Kasperek EM, Zhu C, et al. Immune-intrinsic Myd88 directs the production of antibodies with specificity for extracellular matrix components in primary Sjögren's syndrome. *Front Immunol*. 2021;12, 692216. https://doi.org/10.3389/fimmu.2021.692216.

54 Gao R, Tang J, Dong Y, et al. The aberrant levels of decorin induce damages of human salivary gland epithelial cells and polarization of macrophages. *Mod Rheumatol*. 2021;33(3):557–565. https://doi.org/10.1093/mr/roac034.

55 Zhang X, Yun JS, Han D, Yook JI, Kim HS, Cho ES. TGF-β pathway in salivary gland fibrosis. *Int J Mol Sci*. 2020;21(23):9138. https://doi.org/10.3390/ijms21239138.

56 Cheng SCH, Wu VWC, Kwong DLW, Ying MTC. Assessment of post-radiotherapy salivary glands. *Br J Radiol*. 2011;84(1001):393–402. https://doi.org/10.1259/bjr/66754762.

57 Friedrich RE, Bartel-Friedrich S, Holzhausen HJ, Lautenschläger C. The effect of external fractionated irradiation on the distribution pattern of extracellular matrix proteins in submandibular salivary glands of the rat. *J Cranio Maxill Surg*. 2002;30(4):246–254. https://doi.org/10.1054/jcms.2002.0318.

58 Nam K, Maruyama CL, Trump BG, et al. Post-irradiated human submandibular glands display high collagen deposition, disorganized cell junctions, and an increased number of adipocytes. *J Histochem Cytochem*. 2016;64(6):343–352. https://doi.org/10.1369/0022155416646089.

59 Sørensen CE, Larsen JO, Reibel J, et al. Associations between xerostomia, histopathological alterations, and autonomic innervation of labial salivary glands in men in late midlife. *Exp Gerontol*. 2014;57:211–217. https://doi.org/10.1016/j.exger.2014.06.004.

60 Nanduri LSY, Lombaert IMA, Zwaag M, et al. Salisphere derived c-Kit+ cell transplantation restores tissue homeostasis in irradiated salivary gland. *Radiother Oncol*. 2013;108(3):458–463. https://doi.org/10.1016/j.radonc.2013.05.020.

61 Ingle Y, Sarode GS, Sarode SC. A novel histological grading system for minor salivary gland involvement in oral submucous fibrosis patients with clinico-pathological validation. *J Oral Biol Craniofacial Res*. 2021;11(4):596–600. https://doi.org/10.1016/j.jobcr.2021.09.003.

62 Leehan KM, Pezant NP, Rasmussen A, et al. Fatty infiltration of the minor salivary glands is a selective feature of aging but not Sjögren's syndrome. *Autoimmunity*. 2017;50(8):451–457. https://doi.org/10.1080/08916934.2017.1385776.

63 Klein A, Klein J, Chacham M, et al. Acinar atrophy, fibrosis and fatty changes are significantly more common than Sjogren's syndrome in minor salivary gland biopsies. *Medicina*. 2022;58(2):175. https://doi.org/10.3390/medicina58020175.

64 Nery LR, Moreira CR, Cestari TM, Taga R, Damante JH. Postmortem acinar autolysis in rat sublingual gland: a morphometric study. *J Appl Oral Sci*. 2010;18(5):509–514. https://doi.org/10.1590/s1678-77572010000500014.

65 Choi JS, Park IS, Kim S ki, Lim JY, Kim YM. Analysis of age-related changes in the functional morphologies of salivary glands in mice. *Arch Oral Biol*. 2013;58(11):1635–1642. https://doi.org/10.1016/j.archoralbio.2013.07.008.

66 Yamauchi Y, Matsuno T, Omata K, Satoh T. Relationship between hyposalivation and oxidative stress in aging mice. *J Clin Biochem Nutr*. 2017;61(1):40–46. https://doi.org/10.3164/jcbn.16-79.

67 Tomisaki H, Sekine J, Inokuchi T, Izumi SI, Takano K. Immunohistochemical identification of HSP47 and Type I procollagen-expressing cells in Mongolian gerbil salivary glands: correlation with age-related fibrotic changes. *Oral Medicine Pathology*. 2004;9(4):141–148. https://doi.org/10.3353/omp.9.141.

68 Westbury CB, Yarnold JR. Radiation fibrosis — current clinical and therapeutic perspectives. *Clin Oncol*. 2012;24(10):657–672. https://doi.org/10.1016/j.clon.2012.04.001.

69 Avila JL, Grundmann O, Burd R, Limesand KH. Radiation-induced salivary gland dysfunction results from p53-dependent apoptosis. *Int J Radiat Oncol Biol Phys*. 2009;73(2):523–529. https://doi.org/10.1016/j.ijrobp.2008.09.036.

70 Grundmann O, Mitchell GC, Limesand KH. Sensitivity of salivary glands to radiation: from animal models to therapies. *J Dent Res*. 2009;88(10):894–903. https://doi.org/10.1177/0022034509343143.

71 Morgan-Bathke M, Hill GA, Harris ZI, et al. Autophagy correlates with maintenance of salivary gland function following radiation. *Sci Rep-uk*. 2014;4(1):5206. https://doi.org/10.1038/srep05206.

72 Radfar L, Sirois DA. Structural and functional injury in minipig salivary glands following fractionated exposure to 70 Gy of ionizing radiation: an animal model for human radiation-induced salivary gland injury. *Oral Surg Oral Medicine Oral Pathology Oral Radiology Endodontology*. 2003;96(3):267–274. https://doi.org/10.1016/s1079-2104(03)00369-x.

73 Teos LY, Zheng CY, Liu X, et al. Adenovirus-mediated hAQP1 expression in irradiated mouse salivary glands causes recovery of saliva secretion by enhancing acinar cell volume decrease. *Gene Ther*. 2016;23(7):572–579. https://doi.org/10.1038/gt.2016.29.

74 Friedrich RE, Bartel-Friedrich S, Röser K, Lautenschläger C. The expression pattern of collagen I in irradiated mandibular salivary glands of rats. *Anticancer Res*. 2003;23(2A):927–930.

75 Wu VWC, Leung KY. A Review on the assessment of radiation induced salivary gland damage after radiotherapy. *Front Oncol*. 2019;9:1090. https://doi.org/10.3389/fonc.2019.01090.

76 Luitje ME, Israel AK, Cummings MA, et al. Long-term maintenance of acinar cells in human submandibular glands after radiation therapy. *Int J Radiat Oncol Biol Phys*. 2021;109(4):1028–1039. https://doi.org/10.1016/j.ijrobp.2020.10.037.

77 Liu X, Ong HL, Ambudkar I. TRP channel involvement in salivary glands—some good, some bad. *Cells*. 2018;7(7):74. https://doi.org/10.3390/cells7070074.

78 Sisto M, Ribatti D, Lisi S. SMADS-mediate molecular mechanisms in Sjögren's syndrome. *Int J Mol Sci*. 2021;22(6):3203. https://doi.org/10.3390/ijms22063203.

79 Hakim SG, Ribbat J, Berndt A, et al. Expression of Wnt-1, TGF-β and related cell–cell adhesion components following radiotherapy in salivary glands of patients with manifested radiogenic xerostomia. *Radiother Oncol*. 2011;101(1):93–99. https://doi.org/10.1016/j.radonc.2011.07.032.

80 Spiegelberg L, Swagemakers SMA, Ijcken WFJ, et al. Gene expression analysis reveals inhibition of radiation-induced TGFβ-signaling by hyperbaric oxygen therapy in mouse salivary glands. *Mol Med*. 2014;20(1):257–269. https://doi.org/10.2119/molmed.2014.00003.

81 Budi EH, Schaub JR, Decaris M, Turner S, Derynck R. TGF-β as a driver of fibrosis: physiological roles and therapeutic opportunities. *J Pathol*. 2021;254(4):358–373. https://doi.org/10.1002/path.5680.

82 Xuan J, Shen L, Malyavantham K, Pankewycz O, Ambrus JL, Suresh L. Temporal histological changes in lacrimal and major salivary glands in mouse models of Sjogren's syndrome. *BMC Oral Health*. 2013;13(1):51. https://doi.org/10.1186/1472-6831-13-51.

83 Barone F, Campos J, Bowman S, Fisher BA. The value of histopathological examination of salivary gland biopsies in diagnosis, prognosis and treatment of Sjögren's Syndrome. *Swiss Med Wkly*. 2015;145(3738), w14168. https://doi.org/10.4414/smw.2015.14168.

84 Chen X, Wu H, Wei W. Advances in the diagnosis and treatment of Sjogren's syndrome. *Clin Rheumatol*. 2018;37(7):1743–1749. https://doi.org/10.1007/s10067-018-4153-8.

85 Bombardieri M, Argyropoulou OD, Ferro F, et al. One year in review 2020: pathogenesis of primary Sjögren's syndrome. Suppl 126 *Clin Exp Rheumatol*. 2020;38(4):3–9.

86 Sisto M, Lorusso L, Ingravallo G, Tamma R, Ribatti D, Lisi S. The TGF-β1 signaling pathway as an attractive target in the fibrosis pathogenesis of Sjögren's syndrome. *Mediat Inflamm*. 2018;2018, 1965935. https://doi.org/10.1155/2018/1965935.

87 Sisto M, Lorusso L, Tamma R, Ingravallo G, Ribatti D, Lisi S. Interleukin-17 and -22 synergy linking inflammation and EMT-dependent fibrosis in Sjögren's syndrome. *Clin Exp Immunol*. 2019;198(2):261–272. https://doi.org/10.1111/cei.13337.

88 Skopouli FN, Li L, Boumba D, et al. Association of mast cells with fibrosis and fatty infiltration in the minor salivary glands of patients with Sjögren's syndrome. *Clin Exp Rheumatol*. 1998;16(1):63–65.

89 Bookman AAM, Shen H, Cook RJ, et al. Whole stimulated salivary flow: correlation with the pathology of inflammation and damage in minor salivary gland biopsy specimens from patients with primary Sjögren's syndrome but not patients with sicca. *Arthritis Rheum*. 2011;63(7):2014–2020. https://doi.org/10.1002/art.30295.

90 Molina C, Alliende C, Aguilera S, et al. Basal lamina disorganisation of the acini and ducts of labial salivary glands from patients with Sjögren's syndrome: association with mononuclear cell infiltration. *Ann Rheum Dis*. 2006;65(2):178. https://doi.org/10.1136/ard.2004.033837.

91 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 Rheum*. 2004;50(12):3968–3973. https://doi.org/10.1002/art.20730.

92 Porola P, Mackiewicz Z, Laine M, et al. Laminin isoform profiles in salivary glands in Sjögren's syndrome. *Adv Clin Chem*. 2011;55:35–59. https://doi.org/10.1016/b978-0-12-387042-1.00003-4.

93 Konttinen YT, Porola P, Konttinen L, Laine M, Poduval P. Immunohistopathology of Sjögren's syndrome. *Autoimmun Rev*. 2006;6(1):16–20. https://doi.org/10.1016/j.autrev.2006.03.003.

94 Mao XD, Min SN, Zhu MQ, et al. The role of endothelial barrier function in the fibrosis of salivary gland. *J Dent Res*. 2023;102(1):82–92. https://doi.org/10.1177/00220345221118508.

95 Tanaka J, Mishima K. Application of regenerative medicine to salivary gland hypofunction. *Jpn Dent Sci Rev*. 2021;57:54–59. https://doi.org/10.1016/j.jdsr.2021.03.002.

96 Coppes RP, Meter A, Latumalea SP, Roffel AF, Kampinga HH. Defects in muscarinic receptor-coupled signal transduction in isolated parotid gland cells after in vivo irradiation: evidence for a non-DNA target of radiation. *Br J Cancer*. 2005;92(3):539–546. https://doi.org/10.1038/sj.bjc.6602365.

97 Alevizos I, Zheng C, Cotrim A, et al. Immune reactivity after adenoviral-mediated aquaporin-1 cDNA transfer to human parotid glands. *Oral Dis*. 2017;23(3):337–346. https://doi.org/10.1111/odi.12614.

98 Parisis D, Chivasso C, Perret J, Soyfoo MS, Delporte C. Current state of knowledge on primary Sjögren's syndrome, an autoimmune exocrinopathy. *J Clin Medicine*. 2020;9(7):2299. https://doi.org/10.3390/jcm9072299.

99 Marinkovic M, Tran ON, Wang H, et al. Autologous mesenchymal stem cells offer a new paradigm for salivary gland regeneration. *Int J Oral Sci*. 2023;15(1):18. https://doi.org/10.1038/s41368-023-00224-5.

100 Chowdhury EA, Meno-Tetang G, Chang HY, et al. Current progress and limitations of AAV mediated delivery of protein therapeutic genes and the importance of developing quantitative pharmacokinetic/pharmacodynamic (PK/PD) models. *Adv Drug Deliv Rev*. 2021;170:214–237. https://doi.org/10.1016/j.addr.2021.01.017.

101 Hajiabbas M, D'Agostino C, Simińska-Stanny J, Tran SD, Shavandi A, Delporte C. Bioengineering in salivary gland regeneration. *J Biomed Sci*. 2022;29(1):35. https://doi.org/10.1186/s12929-022-00819-w.

102 Peng X, Wu Y, Brouwer U, et al. Cellular senescence contributes to radiation-induced hyposalivation by affecting the stem/progenitor cell niche. *Cell Death Dis*. 2020;11(10):854. https://doi.org/10.1038/s41419-020-03074-9.

103 Wang X, Bootsma H, Koning J, Kroese FGM, Pringle S. Novel approaches for rescuing function of the salivary gland epithelium in primary Sjögren's syndrome. Suppl 126 *Clin Exp Rheumatol*. 2020;38(4):261–270.

104 Wu Y. *Salivary Gland Organoids to Study Radiation-Induced Microenvironmental Changes: A Versatile Translational Tool [dissertation]*. Groningen (NL): University of Groningen; 2002. https://doi.org/10.33612/diss.240663975. Published online 2022.

105 Walraven M, Hinz B. Therapeutic approaches to control tissue repair and fibrosis: extracellular matrix as a game changer. *Matrix Biol*. 2018;71:205–224. https://doi.org/10.1016/j.matbio.2018.02.020.

106 Agha EE, Kramann R, Schneider RK, et al. Mesenchymal stem cells in fibrotic disease. *Cell Stem Cell*. 2017;21(2):166–177. https://doi.org/10.1016/j.stem.2017.07.011.

107 Marinkovic M, Tran ON, Block TJ, et al. Native extracellular matrix, synthesized ex vivo by bone marrow or adipose stromal cells, faithfully directs mesenchymal stem cell differentiation. *Matrix Biol. Plus. Published online*. 2020, 100044. https://doi.org/10.1016/j.mbplus.2020.100044.

108 Burk J, Sassmann A, Kasper C, Nimptsch A, Schubert S. Extracellular matrix synthesis and remodeling by mesenchymal stromal cells is context-sensitive. *Int J Mol Sci*. 2022;23(3):1758. https://doi.org/10.3390/ijms23031758.

109 Tandon M, Perez P, Burbelo PD, Calkins C, Alevizos I. Laser microdissection coupled with RNA-seq reveal cell-type and disease-specific markers in the salivary gland of Sjögren's syndrome patients. *Clin Exp Rheumatol*. 2016;35(5):777–785.

110 Noll B, Mougeot FB, Brennan MT, Mougeot JLC. Regulation of MMP9 transcription by ETS1 in immortalized salivary gland epithelial cells of patients with salivary hypofunction and primary Sjögren's syndrome. *Sci Rep*. 2022;12(1), 14552. https://doi.org/10.1038/s41598-022-18576-z.

111 Sisto M, D'Amore M, Lofrumento DD, et al. Fibulin-6 expression and anoikis in human salivary gland epithelial cells: implications in Sjogren's syndrome. *Int Immunol*. 2009;21(3):303–311. https://doi.org/10.1093/intimm/dxp001.

112 Sarode SC, Sharma NK, Sarode G, Sharma M, Radhakrishnan R. Targeting the immune-privileged myofibroblast in oral submucous fibrosis by CAR T-cell therapy. *Med Hypotheses*. 2022;165, 110897. https://doi.org/10.1016/j.mehy.2022.110897.

113 Tran ON, Wang H, Li S, et al. Organ-specific extracellular matrix directs trans-differentiation of mesenchymal stem cells and formation of salivary gland-like organoids in vivo. *Stem Cell Res Ther*. 2022;13(1):306. https://doi.org/10.1186/s13287-022-02993-y.

114 Krampera M, Blanc KL. Mesenchymal stromal cells: putative microenvironmental modulators become cell therapy. *Cell Stem Cell*. 2021;28(10):1708–1725. https://doi.org/10.1016/j.stem.2021.09.006.

115 Bui TM, Wiesolek HL, Sumagin R. ICAM-1: a master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. 2020;108(3):787–799. https://doi.org/10.1002/jlb.2mr0220-549r.

116 Lozito TP, Jackson WM, Nesti LJ, Tuan RS. Human mesenchymal stem cells generate a distinct pericellular zone of MMP activities via binding of MMPs and secretion of high levels of TIMPs. *Matrix Biol*. 2014;34:132–143. https://doi.org/10.1016/j.matbio.2013.10.003.

117 Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci*. 2007;48(1):15–24. https://doi.org/10.1016/j.jdermsci.2007.05.018.

118 Chen ZY, Hu YY, Hu XF, Cheng LX. The conditioned medium of human mesenchymal stromal cells reduces irradiation-induced damage in cardiac fibroblast cells. *J Radiat Res*. 2018;59(5), rry048. https://doi.org/10.1093/jrr/rry048.

119 Wang X, Ma Y, Gao Z, Yang J. Human adipose-derived stem cells inhibit bioactivity of keloid fibroblasts. *Stem Cell Res Ther*. 2018;9(1):40. https://doi.org/10.1186/s13287-018-0786-4.

120 Li J, Li Z, Wang S, Bi J, Huo R. Exosomes from human adipose-derived mesenchymal stem cells inhibit production of extracellular matrix in keloid fibroblasts via downregulating transforming growth factor-β2 and Notch-1 expression. *Bioengineered*. 2022;13(4):8515–8525. https://doi.org/10.1080/21655979.2022.2051838.

121 Helm D, Groenewoud A, Jonge-Muller ESM, et al. Mesenchymal stromal cells prevent progression of liver fibrosis in a novel zebrafish embryo model. *Sci Rep-uk*. 2018;8(1), 16005. https://doi.org/10.1038/s41598-018-34351-5.

122 Kore RA, Wang X, Ding Z, Griffin RJ, Tackett AJ, Mehta JL. MSC exosome-mediated cardioprotection in ischemic mouse heart comparative proteomics of infarct and peri-infarct areas. *Mol Cell Biochem*. 2021;476(4):1691–1704. https://doi.org/10.1007/s11010-020-04029-6.

123 Marinkovic M, Block TJ, Kondraske G. Toward a unified "quality" framework for cell-based therapies. *Cytotherapy*. 2018;20(10):1220–1222. https://doi.org/10.1016/j.jcyt.2018.08.003.

124 Nemeth K, Keane-Myers A, Brown JM, et al. Bone marrow stromal cells use TGF-β to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci USA*. 2010;107(12):5652–5657. https://doi.org/10.1073/pnas.0910720107.

125 Ngo MA, Müller A, Li Y, et al. Human mesenchymal stem cells express a myofibroblastic phenotype in vitro: comparison to human cardiac myofibroblasts. *Mol Cell Biochem*. 2014;392(1-2):187–204. https://doi.org/10.1007/s11010-014-2030-6.

126 Wang LT, Ting CH, Yen ML, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *J Biomed Sci*. 2016;23(1):76. https://doi.org/10.1186/s12929-016-0289-5.

127 Mora AL, Rojas M. Aging and lung injury repair: a role for bone marrow derived mesenchymal stem cells. *J Cell Biochem*. 2008;105(3):641–647. https://doi.org/10.1002/jcb.21890.

128 Lai Y, Sun Y, Skinner CM, et al. Reconstitution of marrow-derived extracellular matrix ex vivo: a robust culture system for expanding large-scale highly functional human mesenchymal stem cells. *Stem Cell Dev*. 2010;19(7):1095–1107. https://doi.org/10.1089/scd.2009.0217.

129 Chen XDD. Extracellular matrix provides an optimal niche for the maintenance and propagation of mesenchymal stem cells. *Birth Defects Res. C Embryo Today*. 2010;90(1):45–54. https://doi.org/10.1002/bdrc.20171.

130 Block TJ, Marinkovic M, Tran ON, et al. Restoring the quantity and quality of elderly human mesenchymal stem cells for autologous cell-based therapies. *Stem Cell Res Ther*. 2017;8(1):239. https://doi.org/10.1186/s13287-017-0688-x.

131 Majd H, Wipff P, Buscemi L, et al. A novel method of dynamic culture surface expansion improves mesenchymal stem cell proliferation and phenotype. *Stem Cell*. 2009;27(1):200–209. https://doi.org/10.1634/stemcells.2008-0674.

132 Talele NP, Fradette J, Davies JE, Kapus A, Hinz B. Expression of α-smooth muscle actin determines the fate of mesenchymal stromal cells. *Stem Cell Rep*. 2015;4(6):1016–1030. https://doi.org/10.1016/j.stemcr.2015.05.004.

133 Marinkovic M, Block TJ, Rakian R, et al. One size does not fit all: developing a cell-specific niche for in vitro study of cell behavior. *Matrix Biol*. 2016;52–54:426–441. https://doi.org/10.1016/j.matbio.2016.01.004.

134 Hoshiba T. Decellularized extracellular matrix for stem cell culture. In: *Engineering Materials for Stem Cell Regeneration. 1st Ed*. Springer; 2021:429–454. https://doi.org/10.1007/978-981-16-4420-7_17. Published online 2021.

135 Sun Y, Li W, Lu Z, et al. Rescuing replication and osteogenesis of aged mesenchymal stem cells by exposure to a young extracellular matrix. *Faseb J*. 2011;25(5):1474–1485. https://doi.org/10.1096/fj.10-161497.

136 Chen XD, Dusevich V, Feng JQ, Manolagas SC, Jilka RL. Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. *J Bone Miner Res*. 2007;22(12):1943–1956. https://doi.org/10.1359/jbmr.070725.

137 Zhang BX, Zhang ZL, Lin AL, et al. Silk fibroin scaffolds promote formation of the ex vivo niche for salivary gland epithelial cell growth, Matrix Formation, and Retention of Differentiated Function. *Tissue Eng*. 2015;21(9-10):1611–1620. https://doi.org/10.1089/ten.tea.2014.0411.

138 Rakian R, Block TJ, Johnson SM, et al. Native extracellular matrix preserves mesenchymal stem cell "stemness" and differentiation potential under serum-free culture conditions. *Stem Cell Res Ther*. 2015;6(1):1–11. https://doi.org/10.1186/s13287-015-0235-6.

139 Li CX, Talele NP, Boo S, et al. MicroRNA-21 preserves the fibrotic mechanical memory of mesenchymal stem cells. *Nat Mater*. 2017;16(3):379–389. https://doi.org/10.1038/nmat4780.