# Title: “Wharton’s Jelly Mesenchymal Stromal Cells to Improve Salivary Secretion in a Sjögren-like Disease Mouse Model”

*Author*: Stephanie-Rachel Sultan

*Supervisor*: Dr. Pierre Laneuville

*Co-Supervisor*: Dr. Simon Tran

Department of Experimental Medicine, McGill University, Montreal
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# LIST OF ABBREVIATIONS*

SS (Sjögren's Syndrome)
MSC (Mesenchymal Stromal Cell)
NOD (Non-Obese Diabetic)
SG (Salivary Gland)
PG (Parotid Gland)
SMG (Submandibular Gland)
SLG (Sublingual Gland)
BM (Bone Marrow)
SFR (Salivary Flow Rate)
IFN (Interferon)
TNF (Tumor Necrosis Factor)
AT (Adipose Tissue)
WT (Wharton Jelly)
DPSC (Dental Pulp Derived Stem Cells)
UC (Umbilical Cord)
IV (Intravenous)
IP (Intraperitoneal)
IG (Intraglandular)
CP (Cord Plasma)
PL (Platelet Lysate)
DIN (Donor Identification Number)
DM (Diabetes Mellitus)
H&E (Hematoxylin and Eosin)

* Includes only abbreviations that appear 3 times or more in-text.

# ABSTRACT

**Introduction:** Sjögren's Syndrome (SS) is a chronic autoimmune disorder primarily characterized by xerostomia (dry mouth) and xerophthalmia (dry eyes), resulting from immune-mediated inflammation of the salivary and lacrimal glands. Current therapeutic options for managing xerostomia in SS patients include sialagogues and artificial saliva substitutes, both of which necessitate frequent administration and provide only temporary relief. Recent studies on mesenchymal stromal cells (MSCs) derived from various tissue sources have shown promise in enhancing salivary gland function. MSCs from adipose tissue (AT) have demonstrated efficacy, with reports of a 33% increase in saliva production after four weeks and a 50% increase after four months in treated patients. Additionally, MSCs derived from bone marrow (BM) have been shown to modulate immune responses by reducing inflammation and autoreactive lymphocyte activity, ultimately restoring salivary gland function in both murine models and SS patients. Wharton's jelly-derived MSCs (WJ-MSCs), harvested from the umbilical cord (UC) — a typically discarded tissue post-childbirth — represent a particularly valuable source of MSCs. WJ-MSCs exhibit high proliferative potential and the capacity to differentiate into adipogenic, osteogenic, and chondrogenic lineages, while also demonstrating low immunogenicity. Their anti-inflammatory properties further enhance their suitability as an allogeneic cell source for stem cell therapies, making them a viable option for transplantation without requiring donor-recipient matching.

**Objectives:** This study investigates the potential of WJ-MSCs as a cell-based therapy for SS-like disease in non-obese diabetic (NOD) mice. The primary objective is to evaluate whether intraglandular administration of WJ-MSCs reduces inflammation and improves saliva production in this model. By assessing these effects, this research aims to provide a deeper understanding of

the therapeutic potential of WJ-MSCs for SS, contributing to the development of more effective treatment strategies for this condition

**Methods:** WJ-MSCs were isolated, expanded, characterized, and cryopreserved from five human individual umbilical cords obtained with informed consent. After thawing, the samples were pooled, with one group of 8-week-old female NOD mice (treatment) receiving WJ-MSCs via non-surgical transcutaneous intraglandular injection into the submandibular gland, while a control group received normal saline. Blood glucose levels were monitored weekly from 11 weeks onward to detect diabetes onset. At 16 weeks, the mice were sacrificed for SG regeneration analyses.

**Results:** Eight weeks post-treatment, the WJ-MSC-treated group exhibited significantly higher salivary flow rates (SFR) compared to the control group. At weeks 12 and 16, the WJ-MSC-treated group demonstrated a significant increase in mean SFR compared to the control (normal saline) group ($p < 0.05$), which maintained consistently low SFRs throughout the study. Histopathological analysis revealed reduced lymphocytic infiltration in the submandibular and sublingual glands of WJ-MSC-treated mice. Focus score analysis demonstrated a significantly lower mean focus score in the treatment group compared to the control group ($p \leq 0.05$).

**Conclusion**: This study suggests that non-surgical intraglandular administration of WJ-MSCs is a promising therapeutic approach for enhancing saliva production and restoring salivary gland function in a SS-like model. The accessibility of WJ-MSCs supports their potential as an "off-the-shelf" treatment, offering a readily available, targeted option to improve patient outcomes and advance the management of autoimmune salivary gland disorders.

# RÉSUMÉ

**Introduction**: Le syndrome de Sjögren (SS) est une maladie auto-immune chronique caractérisé par la xérostomie (bouche sèche) et la xérophtalmie (yeux secs), résultant de l'inflammation médiée par le système immunitaire des glandes salivaires et lacrymales. Les options thérapeutiques actuelles pour la gestion de la xérostomie chez les patients atteints de SS incluent les sialagogues et les substituts de salive artificielle, qui nécessitent une administration fréquente et n'apportent qu'un soulagement temporaire. Des études récentes sur les cellules souches stromales mésenchymateuses (MSC) dérivées de diverses sources tissulaires ont montré des résultats prometteurs pour améliorer la fonction des glandes salivaires. Les MSC issues du tissu adipeux (AT) ont démontré leur efficacité, avec des rapports indiquant une augmentation de 33 % de la production de salive après quatre semaines et une augmentation de 50 % après quatre mois chez les patients traités. De plus, les MSC dérivées de la moelle osseuse (BM) ont montré leur capacité à supprimer les réponses auto-immunes et à restaurer la fonction des glandes salivaires chez les modèles murins et chez les patients atteints de SS. Les MSC dérivées de la gelée de Wharton (WJ-MSC), récoltées à partir du cordon ombilical (UC), un tissu généralement jeté après l'accouchement, représentent une source particulièrement précieuse de MSC. Les WJ-MSC présentent un fort potentiel prolifératif et la capacité de se différencier en lignées adipogéniques, ostéogéniques et chondrogéniques, tout en démontrant une faible immunogénicité. Leurs propriétés anti-inflammatoires renforcent encore leur adéquation en tant que source de cellules transplantables pour les thérapies par cellules souches.

**Objectifs**: Cette étude examine l'utilisation des cellules souches mésenchymateuses dérivées de la gelée de Wharton (WJ-MSC) comme thérapie cellulaire pour le traitement du

syndrome de Sjögren (SS)-like chez des souris diabétiques non obèses (NOD). L'objectif principal est d'évaluer l'efficacité de l'administration intraglandulaire de WJ-MSC pour réduire l'inflammation et augmenter la production de salive dans ce modèle de type SS.

**Méthodes**: Les WJ-MSC humaines ont été isolées, étendues, caractérisées et cryoconservées à partir de cinq cordons ombilicaux individuels obtenus avec consentement éclairé. Après décongélation, les échantillons ont été regroupés, un groupe de souris femelles NOD (n = 8) âgées de 8 semaines (traitement) recevant des WJ-MSC par injection intraglandulaire transcutanée non chirurgicale dans la glande submandibulaire, tandis qu'un groupe témoin (n = 7) recevait une solution saline. Les niveaux de glucose sanguin ont été surveillés chaque semaine à partir de la 11e semaine pour détecter l'apparition du diabète. À 16 semaines, les souris ont été sacrifiées pour des analyses de régénération des glandes salivaires (SG).

**Résultats**: Huit semaines après le traitement, le groupe traité par WJ-MSC a présenté des débits salivaires significativement plus élevés par rapport au groupe témoin. L'analyse histopathologique a montré une infiltration lymphocytaire réduite dans les glandes submandibulaires et sublinguales des souris traitées par WJ-MSC.

**Conclusion**: Cette étude suggère que l'administration intraglandulaire non chirurgicale de WJ-MSC est une approche thérapeutique prometteuse pour améliorer la production de salive et restaurer la fonction des glandes salivaires dans ce modèle de syndrome de Sjögren. L'accessibilité des WJ-MSC soutient leur potentiel en tant que traitement "prêt-à-l'emploi", offrant une option ciblée et disponible pour améliorer les résultats chez les patients et faire progresser la prise en charge des troubles auto-immuns des glandes salivaires.

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# PREFACE AND CONTRIBUTIONS OF AUTHORS

Stephanie-Rachel Sultan was responsible for the isolation, expansion, characterization, cryopreservation and thawing of WJ-MSCs, as well as the preparation and maintenance of all cell cultures and WJ-MSC treatments. She also designed and coordinated the injections for NOD mice, managed the collection of blood samples for glucose monitoring, and performed the measurement of salivary flow rates.

Dr. Janaki Iyer conducted all non-surgical, transcutaneous intraglandular injections and provided supervision and assistance in the collection of blood for glucose monitoring and salivary flow rate measurements. Dr. Iyer was also responsible for the euthanasia of all mice via cardiac puncture and the subsequent collection of tissue samples for salivary gland analysis. In collaboration with Dr. You Nan Liu, Dr. Iyer supervised the data collection, statistical analyses, and interpretation of results.

All chapters of this thesis were written by Stephanie-Rachel Sultan and reviewed and edited by Dr. Pierre Laneuville and Dr. Tran.

# 1. Introduction

## 1.1 Sjogren's Syndrome

Sjögren's Syndrome (SS) is a chronic autoimmune disease characterized by the inflammation and progressive damage, including fibrosis, of the salivary and lacrimal glands, leading to the primary symptoms of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Despite extensive research, the exact cause of SS remains elusive, yet it is generally attributed to an abnormal immune response in glandular tissues, reducing their ability to produce saliva and tears. The pathophysiology of SS involves a complex interplay among genetic, environmental, and hormonal factors, culminating in a self-perpetuating cycle of inflammation and glandular destruction. Histopathological evaluation of tissues from patients with Sjögren Syndrome (SS) reveal dense focal infiltrates predominantly consisting of lymphocytes. These infiltrates are characterized by activated CD4+ helper T cells, along with B cells and plasma cells. he CD4+ helper T cells are particularly significant for their secretion of various cytokines, including interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which play key roles in driving the inflammatory process and tissue damage. These cytokines play a critical role in stimulating antigen-presenting activities within epithelial cells. This antigen presentation is vital for the activation of other immune cells and perpetuation of the autoimmune response. Additionally, the IFN-mediated signaling contributes to the induction of programmed cell death, primarily through the upregulation of the Fas protein, which is instrumental in the apoptotic processes observed in the affected glandular tissues. The B cells within these infiltrates are stimulated by the cytokine environment to differentiate into plasma cells, which then produce autoantibodies such as Anti-Ro/SSA and Anti-La/SSB. These autoantibodies can form immune complexes that contribute to further inflammation and tissue damage.

While primary SS can affect individuals of all ages and genders, it is more frequently diagnosed in women, with a prevalence that is nine times higher than in men. The incidence of SS generally increases with age, most diagnosed in individuals between the ages of 40 and 60 years, although it can also present in pediatric cases. Globally, the prevalence varies but is estimated to affect approximately 0.1% to 0.6% of the population, reflecting differences in diagnostic criteria and studied population ([4], [5]). The impact of SS on quality of life is profound; the persistent dryness complicates basic daily activities such as speaking, swallowing, and maintaining oral hygiene, leading to increased dental decay and oral infections. Eye dryness can result in severe discomfort and visual impairment [3]. Additionally, approximately 50% of SS patients exhibit systemic manifestations including joint pain, skin rashes, and internal organ involvement, further diminishing their quality of life and exacerbating the clinical and economic impacts of the disease.

The etiology of Sjögren's Syndrome (SS) remains unclear despite extensive research, and the condition currently lacks a cure. Management of SS primarily focuses on symptom control rather than curing the disease. Treatment approaches include preventive measures, symptomatic treatments, and systemic therapies. Symptomatic management is essential, with sialagogues commonly used to stimulate saliva production and alleviate dry mouth [71]. Artificial saliva and mucosal moisturizers are frequently employed to manage dry mouth and oral discomfort. Anti-inflammatory medications, such as non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids, are used to reduce glandular swelling and inflammation.

Systemic therapies have evolved, and biologics like rituximab have shown efficacy in reducing B cell infiltration in salivary glands, specifically targeting the lymphocytic infiltration that

characterizes SS. Rituximab has been shown to reduce both the total number and proportion of infiltrating B cells in both minor and major salivary glands [72]. Additionally, combination therapies, such as belimumab plus rituximab, have demonstrated enhanced salivary gland B cell depletion compared to monotherapies, potentially leading to improved clinical outcomes [72]. Despite these advances, no treatment currently halts the progression of the autoimmune response or fully prevents glandular damage. Recent research into the immunomodulatory potential of mesenchymal stromal cells (MSCs) offers promising avenues for more effective therapies, highlighting the ongoing quest for novel treatments that address both the symptoms and the underlying pathophysiology of SS [73].

### 1.2 Salivary Glands

The salivary glands (SGs) play a vital role in maintaining the health and homeostasis of both the oral cavity and the body. By producing and secreting saliva, these glands contribute significantly to oral and systemic health, performing essential functions such as lubrication, pH regulation, and aiding in tooth mineralization. Saliva is a complex biological fluid composed of water, electrolytes (sodium, potassium, calcium, bicarbonate), enzymes (amylase, lysozyme, lipase), antimicrobial proteins (lactoferrin, immunoglobulins), mucins, and growth factors. These components work synergistically to promote digestion, protect oral tissues from infection, lubricate the mouth, and support essential functions like speaking and swallowing. Furthermore, saliva helps cleanse the oral cavity, regulate microbial balance, and maintain the integrity of the gums and teeth, underscoring its crucial role in oral homeostasis. [7].

In patients with autoimmune diseases such as SS, reduced saliva production, or hyposalivation, has a detrimental impact on oral health. This condition leads to taste impairment, difficulties in mastication, deglutition, and articulation, dental deterioration, disruption of oral microbial equilibrium, increased prevalence of oral lesions and infections, gingival disease, and periodontitis. These oral health issues can profoundly impact overall health, potentially leading to malnutrition, while the persistent dysfunction of the salivary glands often results in psychological distress and a diminished quality of life in patients. The functionality of salivary glands is directly correlated with overall quality of life, underscoring the critical importance of maintaining optimal salivary gland function for oral health and daily well-being [8].

The autonomic nervous system plays a critical role in regulating the process of salivary secretion and is responsible for the control of both parasympathetic and sympathetic nerves that provide innervation to the major salivary glands. Parasympathetic nerve fibers from the salivary nucleus in the brainstem travel via the facial and glossopharyngeal nerves to stimulate salivary secretion, leading to increased flow of serous saliva and ions in the salivary glands. The sympathetic nerve fibers increase the production and secretion of proteins by the glands, regulate the blood flow to the glands and modulate local inflammatory and immune mediators [9].

To fully comprehend the structures and importance of the salivary glands in saliva production, as well as their impact in Sjögren's syndrome, it is essential to understand the intricate roles of the minor salivary glands and the three major salivary glands the parotid (PG), submandibular (SMG), and sublingual (SLG) glands. Each of these glands shares a similar histological structure, comprising acinar cells responsible for saliva production and ductal cells that transport saliva to the oral cavity. The acinar cells produce and store saliva in granules,

releasing it upon stimulation. The ductal system consists of intralobular, interlobular, and extralobular ducts. The intralobular ducts receive secretions from the acini and drain them into the interlobular ducts, which then drain into the extralobular ducts. These largest ducts exit the gland and transport saliva to the oral cavity, regulating its volume, composition, and flow rate [10].

**Minor Salivary Glands**

Minor salivary glands are intricate, microscopic clusters of salivary tissue distributed widely throughout the oral cavity, including the lips, cheeks, tongue, and palate. These glands, numbering around 600-1000 in adults, are smaller than the major salivary glands like the parotid and submandibular glands. Functionally, they predominantly secrete mucous saliva, constituting approximately 70-80% of the total saliva volume produced. This mucous secretion is crucial for maintaining continuous basal saliva flow, which is essential for oral moisture and mucosal integrity. It contributes significantly to the lubrication of oral tissues, facilitating clear speech and efficient swallowing processes. The mucous secretions from minor salivary glands play a pivotal role in forming a protective mucosal barrier across the oral surfaces. This barrier not only enhances the overall viscosity of saliva, aiding in the retention and dispersal of antimicrobial enzymes and proteins, but also acts as the first line of defense against microbial invasion. This dual role significantly influences oral comfort and health, contributing to a stable oral environment that supports normal oral functions and protects against various oral diseases [11].

**Major Salivary Glands**

The parotid gland (PG), situated anterior to the ear, is the largest of the salivary glands, playing an essential role in oral health. This gland predominantly consists of serous acinar cells,

although it may contain accessory mucinous acinar cells. Consequently, the saliva secreted by the parotid gland is primarily serous and aqueous, containing proteins and glycoproteins vital for the protection and digestion of oral tissues. Each serous acinus is encircled by myoepithelial cells, which contract to facilitate the expulsion of secretions. Salivary secretion from the parotid gland is stimulated by taste or mastication via the glossopharyngeal nerve (CN IX). The parotid's main excretory duct, Stensen's duct, extends from the anterior portion of the superficial lobe, courses over the masseter muscle, turns medially to penetrate the buccinator muscle, and opens into the buccal cavity adjacent to the maxillary second molar's buccal mucosa. This anatomical pathway ensures efficient delivery of saliva, aiding in digestion and maintaining oral hygiene [12].

The submandibular gland (SMG), situated under the lower jaw, contains both serous and mucous cells. These cells work together to produce a mixed serous and mucous saliva containing enzymes and glycoproteins vital for digestion and oral hygiene. The SMG is innervated by both the facial nerve (CN VII) and the glossopharyngeal nerve (CN IX). The primary excretory duct of the submandibular gland (SMG), known as Wharton’s duct, facilitates the transport of saliva from the SMG to the oral cavity, allowing its release on both sides of the lingual frenulum [11].

The sublingual gland (SLG), the smallest of the three glands, is located beneath the tongue and primarily consists of mucous cells. These cells produce thick, viscous saliva that lubricates and protects the oral cavity. The SLG is mainly innervated by the facial nerve (CN VII). The sublingual gland (SLG) communicates with the oral cavity through multiple excretory ducts, known as the ducts of Rivinus, which drain the gland. The largest of these, Bartholin's duct, merges with Wharton’s duct at the sublingual caruncle, facilitating the efficient transport of saliva [13].

In Sjögren's syndrome, inflammation can damage the ducts, leading to their obstruction or narrowing. This can result in partial or complete blockage, causing swelling and pain in the affected gland, ultimately impacting saliva production and overall oral health. Understanding these processes underscores the significance of maintaining salivary gland function for overall quality of life [14].

### 1.3 Saliva

The production of saliva is a complex process involving both physical and chemical stimuli. The three pairs of major salivary glands produce 90% of the total volume of saliva, while the remaining 10% is secreted almost continuously by the minor salivary glands. Despite their small volume, the minor salivary glands play a crucial role in moistening, lubricating, and protecting the oral mucosa and teeth, especially during sleep. The total volume of saliva secreted by both major and minor glands is referred to as whole saliva. In an unstimulated state, SMGs produce about two-thirds of the total volume of whole saliva. During stimulation, the PGs generate at least 50% of the total saliva volume in the oral cavity. Meanwhile, SLGs contribute to less than 1% of the total daily salivary volume, regardless of whether they are stimulated or not. Approximately 99% of saliva consists of water, while the remaining 1% is comprised of inorganic and organic components that play important roles in oral protection and function. For instance, salivary proteins contribute to the formation of the Acquire Enamel Pellicle (AEP) [15]. This protein layer serves as both a physical and chemical barrier protecting the teeth from microorganisms that preferentially adhere to the tooth surface and form dental plaque. The occurrence of dental plaque is a crucial factor in the onset of two of the most prevalent oral diseases: dental caries and periodontal disease. Salivary amylase is the most abundant protein in human saliva with potential correlation with oral diseases. Its primary role is to effectively start the process of food digestion

in the mouth preventing starch buildup on teeth. Carbonic anhydrase is a protein involved in regulating the pH of saliva and neutralizing the acid generated by microorganisms in dental plaque [15]. As such, it has been explored as a potential regulator for the advancement of dental caries. Patients with SS exhibit lower levels of salivary amylase and carbonic anhydrase, leading to an elevated risk of dental caries and early tooth decay [16].

The saturation of salivary fluid is determined by its pH, calcium, and phosphate concentrations, which are responsible for the dissolution of dental hard tissue. Human resting saliva is oversaturated with calcium carbonate at a pH of 8.6, while stimulated saliva is oversaturated at a pH of 7.3, preventing demineralization of the enamel. The critical pH below which enamel dissolves, in relation to the calcium and phosphate concentrations in plaque fluid, is approximately 5.5 [17]. Studies show that an increase in calcium and phosphate concentrations above average levels in dental plaque fluid (DPF) is associated with a lower incidence of caries, as these minerals have acid-neutralizing properties [17]. Moreover, a higher flow rate of saliva can reduce demineralization, promote remineralization, and prevent the formation of calculus. In patients with SS, inflamed salivary glands reduce salivary flow, resulting in decreased oral phosphate levels, dry mouth, and infections. This leads to an increased risk of progressive caries, difficulty swallowing, and discomfort in the mouth. Saliva loss and the consequent increase in plaque formation may contribute to the development of calculus and periodontal disease. Saliva's buffering system helps to maintain proper acid-base balance by counteracting plaque pH, which aids in oral clearance and helps preserve the integrity of teeth and prosthetic restorations [18].. The carbonic acid/bicarbonate system in saliva is critical for preventing tooth decay by changing the pH and potentially affecting the resistance of decay-causing bacteria. In patients with SS, reduced bicarbonate levels contribute to a diminished buffering capacity and a lower saliva pH.

Specifically, the pH of saliva in SS patients can drop below 6, becoming more acidic, especially when their unstimulated salivary flow rate is insufficient [19]. As a result, buffering ability may be inadequate when saliva secretion rates are low.

Saliva has emerged as a valuable diagnostic tool for the diagnosis of oral and systemic diseases. It provides insights into the progression of diseases and the response to pharmacotherapy [23]. Monitoring changes in salivary composition through various qualitative and quantitative methods is an effective way to detect exposure to pathogens and chemicals and quantify disease severity. The salivary flow rate measurement, serological tests, and minimal salivary gland biopsies are some of the commonly used diagnostic tests for detecting SS. Salivary proteomics also provides a valuable tool for diagnosing SS, by analyzing several biomarkers concurrently affected by the condition [20]. In a recent study, 28 proteins were examined as salivary biomarkers of SS associated with pathology, minor salivary glands, and inflammation [21]. Using salivary diagnostic tests such as salivary flow rate test (SFR), amylase and lipase tests: they have shown accuracy, efficacy, and economic feasibility, demonstrating their competence in clinical diagnosis and predicting the outcome of periodontal disease [22]. Therefore, understanding the composition of saliva and its role in maintaining dental health is crucial for the development of effective preventive and therapeutic approaches.

### 1.4 Anatomical and Histological Comparisons of Salivary Glands in Humans and Mice

The salivary glands in both humans and mice are located bilaterally in the oral cavity, with major glands such as the parotid, submandibular, and sublingual glands situated in similar anatomical positions relative to the oral structures [25].

| Features | Humans | Mice |
|---|---|---|
| **Parotid Gland** | Largest, primarily serous, located near the ear | Primarily serous, similar anatomical location |
| **Submandibular Gland** | Second largest, mixed serous and mucous, located along the lower jaw | Largest, mixed but predominantly serous, fused with sublingual gland |
| **Sublingual Gland** | Smallest major gland, predominantly mucous, located beneath the tongue | Predominantly mucous, fused with submandibular gland |
| **Minor Salivary Glands** | Scattered throughout oral mucosa, varying histological composition | Scattered throughout oral mucosa, similar but varying distribution |

**Table 1: Comparative overview of the major and minor salivary glands in humans and mice.**
The similarities and differences between human and mice are highlighted, noting that while humans have distinct parotid, submandibular, and sublingual glands, mice have a fused submandibular and sublingual gland. The histological composition, including the presence of

serous and mucous cells, is also compared, demonstrating the relevance of mouse models for studying salivary gland function and pathology in humans [11].

### 1.5 NOD Mouse Model for Sjogren's Syndrome

The NOD (Non-Obese Diabetic) mouse model is extensively utilized in Sjogren's syndrome (SS) research due to its ability to recapitulate crucial human disease characteristics. Female NOD mice exhibit gender-specific histopathological and functional alterations in their salivary glands as they age, characterized by lymphocyte infiltration and progressive glandular dysfunction [27]. This leads to a significant reduction in saliva output, paralleling the clinical manifestations observed in SS patients [27]. Consequently, the NOD mouse model serves as a critical tool for uncovering early disease biomarkers and elucidating the underlying biological and immunological dysregulations associated with SS.

**Genetic Susceptibility**: Genetic susceptibility factors are fundamentally implicated in the pathogenesis of autoimmune features observed in both NOD (Non-Obese Diabetic) mice and patients with Sjogren's syndrome. In NOD mice, specific major histocompatibility complex (MHC) haplotypes have been shown to increase susceptibility to autoimmune diseases, including phenotypes similar to SS. These findings highlight the role of MHC genetic variations in the predisposition to autoimmune conditions [21]. These genetic determinants are crucial in modulating the immune system's response, thereby influencing susceptibility to glandular dysfunction. Correspondingly, SS patients exhibit a genetic predisposition associated with particular human leukocyte antigen (HLA) alleles, which are strongly correlated with an elevated risk of disease development and progression. The congruence in genetic susceptibility between NOD mice and SS patients substantiates the relevance of the NOD mouse model in investigating

the genetic basis of SS and provides a robust framework for the development of targeted therapeutic interventions [14].

**Autoantibody Production:** In NOD mice, a model commonly used to study autoimmune diseases like SS autoantibodies such as anti-SSA/Ro and anti-SSB/La are frequently produced, reflecting an active autoimmune response [28]. Approximately 8% of NOD mice produce anti-Ro52 kD antibodies, and elevated titers of anti-Ro and anti-La autoantibodies are detectable by 8 weeks of age in NOD·H2h4 mice ([74],[75]). In humans, approximately 60–80% of patients with SS produce anti-Ro antibodies, and 40–60% produce anti-La antibodies [28]. These autoantibodies, targeted against ribonucleoprotein complexes, are also prevalent in patients with SS, serving as crucial diagnostic markers [14]. The presence and levels of these antibodies correlate strongly with disease severity and glandular dysfunction, offering valuable insights into the autoimmune mechanisms underlying SS. Their identification and quantification in both animal models and human patients are critical for advancing our understanding of immune dysregulation in SS and for developing targeted diagnostic and therapeutic strategies.

**Lymphocytic Infiltration and Histopathological Changes:** Both NOD mice and individuals diagnosed with Sjogren's syndrome (SS) exhibit pronounced lymphocytic infiltration in their exocrine glands, particularly in the salivary and lacrimal glands. In NOD mice, this infiltration is predominantly characterized by CD4+ and CD8+ T cells, accompanied by B cells, which contribute to glandular inflammation through cytokine secretion and direct cytotoxic effects, ultimately leading to tissue damage [29].This immune cell infiltration in NOD mice mirrors the lymphocytic infiltration observed in SS patients, where histological examinations reveal periductal

and perivascular aggregates of T and B lymphocytes within glandular tissues, indicative of focal lymphocytic sialadenitis [30].

Histopathologically, both NOD mice and SS patients show evident changes in their salivary glands. Aging NOD mice develop lymphocytic infiltrates, glandular atrophy, and a reduction in acinar cells, resulting in compromised gland function [31]. Similarly, SS patients exhibit comparable histopathological features, including focal lymphocytic sialadenitis, acinar cell loss, and fibrosis within their salivary glands [30]. These consistent histopathological findings underscore the suitability of NOD mice as a robust model for studying the progression and therapeutic approaches for SS.

**Clinical Manifestations of Xerostomia and Lacrimal Gland Dysfunction:** Both Sjögren's syndrome patients and NOD mice exhibit pronounced similarities in the clinical manifestations of xerostomia (dry mouth) and impaired lacrimal gland function. In SS, these symptoms arise from autoimmune-mediated destruction of salivary and lacrimal gland tissues, leading to diminished saliva production and inadequate tear secretion [30]. Similarly, NOD mice develop lymphocytic infiltration in both salivary and lacrimal glands, with early signs of glandular dysfunction appearing as early as 8 weeks of age, particularly in the submandibular glands. Lymphocytic infiltration becomes more evident by 12 weeks, and glandular dysfunction, marked by reduced saliva and tear production, is clearly observed by 20 weeks [25]. This autoimmune process involves the infiltration of CD4+ and CD8+ T cells in NOD mice, which secrete pro-inflammatory cytokines including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), exacerbating glandular inflammation and tissue damage [33]. Elevated levels of these pro-inflammatory cytokines contribute to both the local inflammation and the systemic

autoimmune pathology, underscoring the similarity between NOD mice and SS patients. Additionally, studies have shown that specific autoantibodies, such as anti-SSA/Ro, anti-SSB/La, and anti-muscarinic receptor III, are elevated in NOD mice, providing further parallels to the immunological markers seen in SS patients [27-32]. The genetic analysis of NOD mice reveals that while certain loci (idd3 and idd5) are linked to SS-like exocrinopathy, the majority are associated with insulin-dependent diabetes, highlighting the complex genetic factors underlying the disease [33].

Sjogren's syndrome involves a multifaceted autoimmune process that affects glandular function, but its manifestation can vary across different animal models, presenting challenges in fully replicating all aspects of SS pathogenesis and clinical manifestations. Despite these challenges, mouse models remain invaluable for elucidating the underlying mechanisms of the disease. They serve a critical role in facilitating the development of potential therapeutic strategies before advancing to human clinical trials. By mimicking key features such as autoimmune-mediated glandular dysfunction and dysregulated cytokine profiles, these models provide critical insights that advance the understanding of SS and inform translational research efforts aimed at improving patient care and outcomes.

### 1.6 Xerostomia and Therapeutic Strategies in Sjogren's Syndrome

Xerostomia, the persistent sensation of dry mouth, is a hallmark symptom of Sjogren's syndrome. The development of xerostomia in SS reflects a complex interplay of immune-mediated processes and structural changes within the salivary gland microenvironment. Chronic lymphocytic infiltration, resulting in glandular destruction and fibrosis over time, disrupts normal

glandular architecture and impairs salivary secretion, thereby contributing to the dry mouth experienced by patients with Sjogren's syndrome ([28], [30]).

Normal salivary function is intricately regulated by the muscarinic M3 receptor (M3R), which plays a pivotal role in stimulating acinar cells to produce and secrete saliva in response to neural and hormonal signals. Activation of M3R by acetylcholine triggers a cascade of intracellular events, including calcium influx and subsequent fluid secretion [28]. In SS, autoimmune processes lead to the production of autoantibodies targeting specific components of salivary gland tissue, such as anti-SSA/Ro and anti-SSB/La antibodies. These autoantibodies form immune complexes within the glandular tissue, triggering chronic inflammation and further disrupting the normal function of M3R-mediated signaling pathways. This disruption impairs the ability of acinar cells to respond to acetylcholine, reducing the secretion of saliva and contributing to the dry mouth symptoms experienced by SS patients [34].

Therapeutic strategies for managing xerostomia in SS primarily aim to alleviate symptoms and improve oral health. Current treatments include patient education on oral hygiene practices, lifestyle modifications to minimize dryness exacerbating factors, and the use of saliva substitutes to maintain moisture in the oral cavity [35]. Pharmacological interventions such as pilocarpine and cevimeline, which stimulate saliva production through M3R activation, are commonly prescribed but may be limited by side effects such as sweating, gastrointestinal disturbances, and cholinergic effects [36]. Despite these therapeutic efforts, there remains a significant unmet need for more effective and sustainable treatments for xerostomia in SS. Immunosuppressive agents, including corticosteroids and biologics targeting B-cell and cytokine pathways, have shown promise in reducing glandular inflammation and preserving function in severe cases of SS-related xerostomia

[37], [38]. However, their use is often associated with potential systemic side effects and requires careful monitoring.

### 1.7 Regenerative Medicine

Emerging research into regenerative medicine approaches holds potential for restoring salivary gland function in SS by promoting tissue repair and regeneration. Techniques such as stem cell therapy and tissue engineering aim to replace damaged glandular tissue and restore physiological saliva production, offering new avenues for long-term management of xerostomia in SS [39]. While significant progress has been made in understanding the pathogenesis of xerostomia in SS and developing symptomatic treatments, ongoing research efforts are essential to address the complex immune mechanisms underlying glandular dysfunction and to advance novel therapeutic strategies that offer durable relief and improve quality of life for SS patients.

### 1.8 Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) are multipotent progenitor cells with the ability to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes. These cells are characterized by their capacity for self-renewal, their potential to modulate immune responses, and their ability to promote tissue repair and regeneration. MSCs can be derived from various tissues, including bone marrow, adipose tissue, dental pulp and umbilical cord blood, each offering unique advantages and challenges in terms of collection, isolation, and expansion. Due to their regenerative properties, MSCs are increasingly recognized as a promising tool in regenerative medicine, with their therapeutic potential extending across a wide range of diseases and conditions. They contribute to tissue healing through paracrine signaling and direct cell replacement, while

their immunomodulatory properties help mitigate inflammation and foster a healing environment [40]. This section explores the diverse sources of MSCs, comparing the methods of collection and their respective benefits, challenges, and clinical applications

**Sources of Mesenchymal Stromal Cells:** MSCs can be isolated from a variety of adult tissues, each offering different collection and expansion methods. The most common sources of MSCs include bone marrow, adipose tissue, and umbilical cord tissue, though they can also be obtained from dental pulp and others. The following table summarizes the most widely used sources of MSCs and their respective advantages, methods of collection, and the challenges associated with each source.

| Source | Collection Method | Advantages | Challenges |
|---|---|---|---|
| **Bone Marrow** | Aspiration from iliac crest or sternum | Well-established source, strong differentiation potential | Invasive collection, limited cell yield, donor site morbidity |
| **Adipose Tissue** | Liposuction or needle biopsy | Abundant, minimally invasive, easier and faster collection, higher MSC yield | Lower quality cells in elderly or obese donors, risk of infection |
| **Umbilical Cord Tissue** | Non-invasive collection during childbirth | Non-invasive, abundant source, low immunogenicity, high proliferative capacity | Limited access, regulatory constraints |
| **Dental Pulp** | Tooth extraction, typically from deciduous teeth | Easily accessible, non-invasive, rich in MSCs | Limited supply, typically requires extraction of healthy teeth |

**Table 2: Comparison of MSC sources based on collection methods, advantages, and challenges.** Bone marrow has strong differentiation but is invasive [75]. Adipose tissue is abundant and easy to collect, though cell quality may vary [76]. Umbilical cord tissue is non-invasive with high proliferative potential but faces regulatory issues [77]. Dental pulp is accessible yet limited in supply [78].

**Characteristics of Mesenchymal Stromal Cells:** While mesenchymal stromal cells (MSCs) exhibit variations depending on their tissue of origin, they must adhere to the core criteria defined by the International Society for Cellular Therapy (ISCT). These updated guidelines, established in 2024, ensure consistency, standardization, and reliability across MSC research, which is essential for advancing the field [41]. However, variations in methods used for the isolation, expansion, and characterization of MSCs have led to inconsistencies across studies, complicating comparisons and impeding progress. To address this challenge, the ISCT's Mesenchymal and Tissue Stem Cell Committee has set minimum guidelines for defining human MSCs, which are crucial for ensuring quality and reproducibility in MSC research. These criteria help enable more accurate, comparable findings across diverse studies and clinical settings.

The updated ISCT criteria specify that MSCs must meet several key requirements:

1. Adherence to plastic: MSCs must adhere to plastic under standard culture conditions.
2. Surface markers: MSCs should express specific surface antigens, including CD105, CD73, and CD90, while lacking the expression of CD45, CD34, CD14 or CD11b, CD19, and HLA-DR.
3. Differentiation capacity: MSCs must demonstrate the ability to differentiate into mesodermal cell types, such as chondrocytes, adipocytes, and osteoblasts, when cultured

under defined conditions. This differentiation potential underscores their crucial role in tissue regeneration.

These criteria are critical for advancing the clinical application of MSCs, as they not only ensure their functional characterization but also highlight their promise in modulating immune responses and promoting tissue repair. This dual functionality makes MSCs a significant therapeutic tool for treating complex conditions and developing novel therapeutic strategies [41].

**Tissue Growth and Repair:** Mesenchymal cells (MSCs) play a vital role in tissue regeneration by producing a variety of bioactive molecules—growth factors and cytokines—that actively stimulate cellular repair mechanisms. Key molecules like VEGF, bFGF, and HGF support cell survival, proliferation, and angiogenesis, which promotes blood vessel formation. Additionally, MSCs secrete neurotrophic factors such as NGF, BDNF, and GDNF, which support nerve growth and repair. This dual secretion fosters an environment conducive to both vascular and neural regeneration, especially in complex tissue remodeling. By mobilizing endothelial progenitors to aid new blood vessel formation and sustaining hematopoietic stem cells through niche-specific factors like SDF-1α, MSCs coordinate vascular and neural growth at injury sites [42].

These regenerative properties position MSCs as a powerful tool in clinical applications, especially for injuries or degenerative diseases where a synergistic approach to tissue healing and neural repair is required. Through both vascular and neural support, MSCs offer a promising approach for enhancing tissue repair, making them an essential focus in regenerative medicine [43].

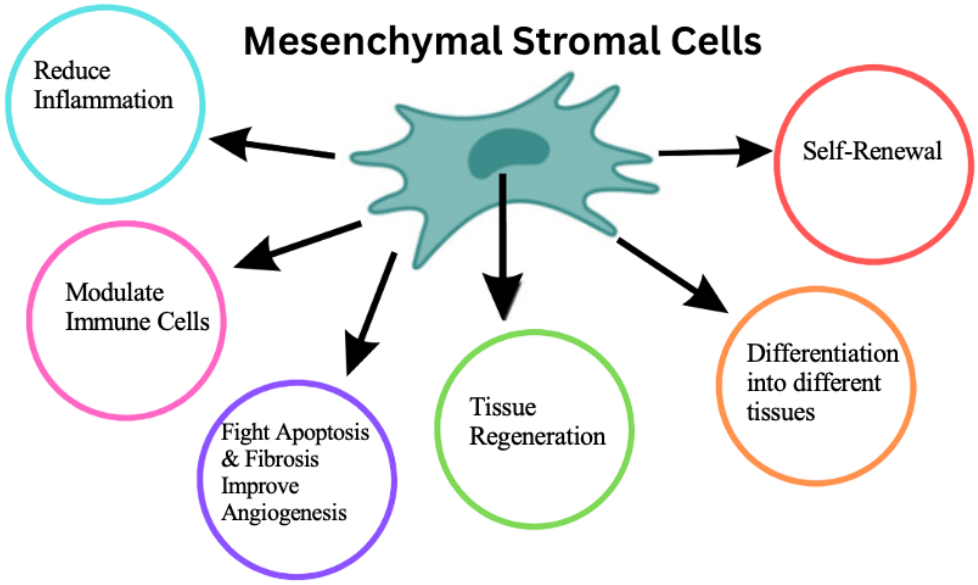



**Figure 1: Key Functional Roles of Mesenchymal Stromal Cells**. MSCs are highly versatile cells that can be isolated from various sources, including bone marrow, adipose tissue, dental pulp, and umbilical cord. These cells offer significant advantages in therapeutic applications due to their ability to differentiate into multiple cell types, promote tissue repair, and modulate immune responses [44]. MSCs are also being investigated for their anti-fibrotic effects, which may be particularly relevant in the context of SS, where excessive fibrosis contributes to glandular dysfunction and impaired salivation. By modulating fibrotic pathways, MSCs could help mitigate the progression of fibrosis in SS. Furthermore, MSCs show promise in cancer therapy by inducing apoptosis in tumor cells and stimulating angiogenesis, which may enhance tumor response to treatment and improve tissue regeneration [88]. *Illustration by Stephanie-Rachel Sultan*.

**Anti-Inflammation Mechanisms**: MSCs possess remarkable immunomodulatory capabilities, enabling them to adjust their response based on the intensity and type of inflammatory signals in their environment. In the early stages of inflammation, MSCs detect pro-inflammatory

signals, such as IL-1β, IFN-γ, TNF-α, and activation of Toll-like receptors, through specific receptors. This activation induces MSCs to release chemokines like CXCL9, CXCL10, CCL5, and macrophage inflammatory protein-1, which stimulate T-cell. At this point, low levels of TNF-α and IFN-γ enhance chemokine secretion without significantly inducing immunosuppressive mediators, such as iNOS in mice or IDO in humans [45].

As inflammation progresses, elevated levels of pro-inflammatory molecules such as IL-1β, IFN-γ, and TNF-α trigger MSCs to shift toward anti-inflammatory responses. MSCs begin secreting TGF-β, IL-10, IDO, and iNOS, which suppress dendritic cell activity, antigen presentation, and T-cell function while promoting regulatory T cell (Treg) proliferation. This shift prevents excessive inflammation and mitigates autoimmune reactions. The transition between pro- and anti-inflammatory effects of MSCs is thought to be regulated by IDO and iNOS levels, functioning as a cellular "switch" that modulates MSC activity based on the inflammatory context [46]. These dual mechanisms allow MSCs to balance immune responses effectively, demonstrating significant therapeutic potential for managing inflammatory and autoimmune diseases. Through their adaptive response to environmental stimuli, MSCs can restore immune homeostasis, making them a promising option in regenerative medicine [47].

**Immunomodulatory Properties**: One of the primary advantages of WJ-MSCs in treating Sjögren's syndrome is their robust immunomodulatory capacity. These cells secrete a range of anti-inflammatory cytokines, which help suppress the overactive immune response typical in SS. By modulating immune cell activity, WJ-MSCs can reduce inflammation and tissue damage, offering a promising therapeutic approach to managing the symptoms of this autoimmune disorder [51]. Studies have demonstrated that WJ-MSCs can significantly downregulate the proliferation

of lymphocytes, alleviating the inflammatory environment within the affected glands [69]. MSCs achieve this by inhibiting the proliferation of T, B, natural killer (NK), and dendritic cells, leading to a state of "division arrest anergy." They also disrupt key immune functions such as cytokine secretion, cytotoxicity, B-cell maturation, antigen presentation, and dendritic cell activation. These effects are particularly potent in inflammatory environments, where MSCs are activated by molecules like TNF-α and IFN-γ. Furthermore, MSCs recruit regulatory T-cells (Tregs) to further suppress immune responses. Soluble factors such as prostaglandin E2, TGF-β, IL-6, and IL-10 contribute to this broad immunomodulation, making it pivotal in managing autoimmune conditions [48].

### 1.9 Previous Studies Utilizing MSCs for Sjogren's Syndrome

MSCs have shown promise in reducing inflammation and restoring the function of irradiated salivary glands in animal models, a concept further supported by human studies. In a more recent study by Xie et al. (2021), the therapeutic potential of MSCs in alleviating Sjögren's syndrome was explored, focusing on their immunomodulatory effects and ability to repair damaged salivary glands. The study found that MSCs significantly reduced inflammation in the salivary glands of SS patients, as evidenced by a marked decrease in pro-inflammatory cytokines such as TNF-α and IL-6. Additionally, the study reported a 50% improvement in salivary flow rate following MSC treatment, with saliva production increasing from a baseline of 0.3 mL/min to 0.45 mL/min after three months of therapy. The authors also observed a significant restoration of tissue structure within the salivary glands, with histological analysis revealing reduced fibrosis and improved acinar cell density. Furthermore, MSCs were shown to modulate the immune response, decreasing the presence of autoreactive T cells and regulatory T cell imbalance, which are commonly

observed in SS. These findings provide robust clinical data that MSCs can significantly improve glandular function and alleviate the debilitating oral complications associated with SS. The incorporation of such human data strengthens the argument for MSC-based therapies, suggesting a viable therapeutic option for SS patients, particularly in addressing the chronic oral symptoms that are central to the disease's impact on quality of life [50].

***Adipose tissue-derived MSCs***

Adipose tissue-derived MSCs and bone marrow-derived MSCs are the most frequently used sources of MSCs for Sjogren's syndrome. One of the early studies in this area examined the therapeutic potential of adipose tissue-derived MSCs in a NOD mouse model of Sjogren's syndrome and reported that this treatment improved salivary gland function and reduced inflammation in the mice [52]. Since then, several other investigate the use of adipose tissue MSCs for Sjogren's syndrome. This tissue source can be obtained either through needle biopsy or liposuction aspiration, and subsequent processing involves the use of collagenase to isolate approximately 5% of the cells contained in the tissue, which results in a yield of approximately 5x103 MSCs per gram [53]. In addition to inflammation reduction, AT-MSCs have the capability of restoring salivary gland function damaged by radiation therapy, as evidenced by *Lynggaard et al.* that increased saliva flow rate and improved blood flow [54] For instance, intraglandular treatment of autologous and allogeneic of human AT-MSCs into the PGs and SMGs enhance the rate of unstimulated salivary flow. At day 120, the unstimulated whole-saliva flow increased from the baseline value of $0.13 \pm 0.02$ mL/min to $0.18 \pm 0.02$ mL/min. Similarly, the stimulated whole-saliva flow rate also increased from $0.66 \pm 0.11$ mL/min to $0.75 \pm 0.11$ mL/min at day 120. Following the administration of AT-MSCs to the parotid and submandibular glands, increase in

the proportion of 63 proteins that are known to be elevated in stimulated whole saliva [54]. The increase in saliva production, both stimulated and unstimulated, indicated an improvement in the clinical condition, suggesting the beneficial effects of AT-MSC therapy.

***Bone Marrow- derived MSCs***

The second most frequent source of MSCs for Sjogren's syndrome is bone marrow-derived MSCs, representing 0.001 to 0.01% of the mononucleated cells from a bone aspirate. When bone marrow-derived cells were transplanted into SS-like mouse models, saliva secretion was significantly enhanced. This resulted in augmented gland weights, MSC differentiation into acinar cells, as demonstrated by histological analysis showing MSCs expressing acinar cell markers such as amylase and cytokeratin 18, and improved saliva production in these mice. Conclusively, AT and BM-derived cells seem to provide similar results in repairing damaged salivary glands, improving both the histological appearance (enhanced acinar cell regeneration and reduced inflammation) and function of the glands. NOD mice treated with BM-MSCs demonstrated an increase in salivary flow rates, a delay in the secretory functions of salivary glands, and lymphocyte infiltration [55]. It is evident from studies that MSC-treatment has a therapeutic effect on NOD mice with Sjögren's disease-like symptoms. Studies have investigated the use of BM-MSCs for the treatment of SS in human subjects. One systematic review by Chihaby et al. (2021) analyzed several clinical trials involving BM-MSC therapy for SS, including a total of 120 patients across multiple centers. The included patients were primarily diagnosed with primary or secondary SS, with common inclusion criteria such as persistent dry mouth, dry eyes, and evidence of salivary gland dysfunction. The intravenous administration of BM-MSCs in these studies resulted in significant improvement in clinical symptoms, including dry mouth and dry eyes, along with

increased saliva and tear production. The beneficial effects of MSC therapy were observed to persist for up to 6 months post-treatment, with some studies reporting sustained improvements in salivary function and overall symptom relief [51]. These findings suggest that BM-MSCs may offer a long-term therapeutic option for SS patients, though further research is needed to better understand the durability of these effects in larger, more diverse patient populations.

### *Dental Pulp-derived MSCs*

As saliva production is directly linked to the oral cavity, there has been research into the use of dental-derived MSCs for cellular therapy in addressing oral and maxillofacial conditions. The pulp tissue is removed surgically, and cells are expanded from vascular fragments. The growth of mineralized tissue and structures in graft models has been demonstrated by a number of studies using dental pulp stem cells (DPSCs) [2]. A comparison of DPSC and BM-MSC treated SS-like mice models revealed that the DPSC induced more cell proliferation, anti-inflammatory effects, immunomodulatory effects and an increase in stimulated salivary flow rate than the BM-MSC. It has been shown that DPSCs suppress activated T cells more effectively than other groups, resulting in a reduction in inflammation in the submandibular glands of mice treated with DPSCs [1]. Obtaining DPSCs requires an invasive surgical procedure, carrying risks of complications. DPSCs are also subjected to donor variability and can be affected by age and health status of the donor.

### *WJ- MSCs: A Promising Treatment Avenue for Sjogren Syndrome*

The harvesting methods of both AT-MSCs and BM-MSCs are invasive and uncomfortable for patients. Additionally, AT-MSCs and BM-MSCS have a lower yield of MSCs compared to WJ-MSCs, which affect the cells available for therapeutic purposes [56]. DPSCs have a limited

capacity for self-renewal and proliferation compared to WJ-MSCs. These sources of are affected by donor age and health status, which can impact their quality and efficacy [79]. Hence, WJ-MSCs is likely a more favorable source of MSCs for therapeutic use owing to their higher yield and ease of collection. Moreover, WJ-MSCs possess potent immunomodulatory properties, making them an appealing choice for the treatment of autoimmune disorders [57].

**Accessibility and Ethical Considerations:** The umbilical cord (UC) consists of two umbilical arteries and one umbilical vein, which are surrounded by a mucous matrix rich in proteoglycans known as the Wharton-Jelly (WJ), a pivotal source of MSCs [58]. WJ-MSCs are readily accessible, as they can be harvested from umbilical cord tissue, a process that is both straightforward and ethical. Unlike stem cells obtained from embryonic sources, which can raise ethical concerns, WJ-MSCs can be collected without ethical dilemmas. This abundance and ease of access make them an attractive option for regenerative therapies. Furthermore, the availability of umbilical cord tissue means that WJ-MSCs can be obtained in large quantities, facilitating their use in clinical applications and allowing for the establishment of standardized protocols for their isolation and expansion [70].

**Regenerative Potential:** Another significant advantage of WJ-MSCs is their inherent regenerative potential. These cells not only have the capacity to differentiate into various cell types, including those relevant to glandular tissue repair, but they also promote tissue regeneration through paracrine signaling. WJ-MSCs release growth factors and other bioactive molecules that can stimulate the repair of damaged tissues, making them particularly suitable for addressing the dry eye and dry mouth symptoms experienced by SS patients [57]. This regenerative capability

supports the restoration of glandular function and overall health, further enhancing the therapeutic prospects of WJ-MSCs in managing SS.

An MSC source that doesn't necessitate liposuction, surgery, anesthesia, lead us to consider the use of umbilical cord (UC). Intraglandular injection of WJ-MSCs could be beneficial for Sjogren syndrome patients, as the cells are directly delivered to the site of inflammation reduce the need for systemic immunosuppressive therapy, which can have adverse side effects. The use of WJ-MSCs by intravenous infusion has shown improvement in salivary gland function, suppressed disease activity and autoimmunity and significantly reduced autoantibodies in patients with SS. Additionally, overall improvement in symptoms were observed in all patients. Within 2 weeks of cell infusion, all patients with symptoms of dry mouth had an increase in both stimulated and unstimulated salivary flow rates [59].

| Study | MSC Source | Model/Study Design | Sample Size (Patients or Animals) | Key Findings |
|---|---|---|---|---|
| Xu et al. [82] | Allogeneic UC-MSCs | Clinical trial (open label) + NOD mice model | 24 SS patients (clinical). NOD mice (n=15/group) | Improved salivary flow rate, reduced lymphocytic infiltration, modulated T cell responses (↓Th1/Th17, ↑Treg); symptoms improved after multiple infusions |
| Shi et al. [83] | UC-MSCs | Preclinical, NOD mice | NOD mice (n=10/group) | MSCs ameliorated SS by suppressing IL-12 production from dendritic cells, leading |

| | | | | |
|---|---|---|---|---|
| | | | | to reduced autoimmunity and gland damage |
| Yao et al. [84] | UC-MSCs | Preclinical, NOD mice | NOD mice (n=10–12/group) | MSCs improved salivary gland function via IFN-β/IL-27 axis; reduced inflammation, promoted glandular homeostasis |
| Alunno et al. [85] | Microencapsulated WJ-MSCs | In vitro (PBMCs from SS patients) | PBMCs from 10 pSS patients and 10 healthy controls | Reduced pro-inflammatory cytokine production (TNF-α, IFN-γ); increased IL-10; demonstrated strong immunomodulatory potential |

**Table 3: Summary of Clinical Studies Investigating the Use of UC-MSCs for SS**

### 1.10 Administration Methods and Donor Variability in WJ-MSC Therapy for Sjögren's Syndrome

Despite significant advancements in therapy, the optimal routes of administration remain a subject of ongoing research and debate due to their inherent limitations and varying efficacy. The most investigated methods in mice models of Sjogren's syndrome (SS) include intravenous (IV), intraperitoneal (IP), and surgical intraglandular (IG) delivery. Each of these methods presents distinct advantages and challenges that impact their therapeutic potential and clinical applicability.

**Intravenous Administration (IV)*:*** Typically, the lateral caudal vein is preferred for its minimally invasive nature and relative safety. However, IV administration of MSCs presents significant challenges. A substantial portion of the injected cells become sequestered in the lungs

and liver, reducing their availability at target tissues and necessitating higher doses to achieve therapeutic effects [80]. This sequestration not only diminishes efficacy but also introduces safety concerns. MSCs express tissue factor, which can trigger the coagulation cascade upon interaction with blood components, increasing the risk of thromboembolic events such as pulmonary embolism and portal vein thrombosis [81]. These limitations underscore the challenges of systemic MSC delivery and highlight the need for alternative administration strategies to improve therapeutic outcomes.

**Intraperitoneal Administration (IP):** While this method requires higher doses of MSCs, it effectively bypasses the pulmonary filtration system, increasing cell availability at targeted sites. However, it is primarily used in preclinical research, as its invasive nature and risk of peritoneal complications limit its applicability for routine clinical use [61].

**Intraglandular (IG) Surgical Administration*:*** Involves the direct injection of MSCs into the glandular tissues, a highly targeted and localized treatment modality. The IG approach allows for significantly lower MSC dosages compared to IV and IP routes, which may help minimize potential systemic exposure. While current clinical trials do not indicate major safety concerns with IV MSC administration, reducing the required cell dose could still be beneficial in optimizing treatment efficiency and minimizing unnecessary systemic distribution. This targeted delivery method has shown promising results in preclinical studies, suggesting a potential for improved clinical outcomes in patients with SS [54]. In 2013, *Tran et al*. conducted research on the intraglandular delivery of BM-MSCs as a treatment for xerostomia in irradiated mice. This study demonstrated that the IG method, which involves making a conservative horizontal incision in the

neck to access the submandibular salivary gland, required a lower number of BM-MSCs to achieve therapeutic effects similar to those obtained through the intravenous route. This observation has since been validated by additional studies [62].

However, despite its therapeutic potential, the IG approach presents significant challenges. Its invasive nature introduces risks of complications, such as damage to adjacent vital structures, which can result in increased mortality rates in mice. These risks highlight the necessity for meticulous evaluation and refinement of the IG technique to ensure a balance between therapeutic efficacy and safety.

**Non-Surgical Transcutaneous Intraglandular Administration*:*** To tackle the challenge of invasive intraglandular (IG) delivery, Almansoori & all developed an innovative non-surgical transcutaneous IG technique for the submandibular salivary glands [62]. This method was designed to provide a less invasive, localized approach to therapeutic delivery [62]. The development of this technique aimed to create an anatomical landmark-guided method for accurately localizing and targeting the SGs. The procedure began with the removal of hair from the neck region, extending from the chest wall to the lower border of the mandible. A midline was then drawn from the lower lip, passing through the inferior border of the mandible down to the chest wall. An incision was made to expose both the right and left submandibular glands, allowing for precise measurement of the distance between the midline and the center of each gland. This measurement was used to determine the optimal injection site, ensuring accurate and effective delivery of therapeutics directly to the target glands [62]. Hence, this approach is particularly appealing for the administration of WJ-MSCs due to its precision in delivering the cells directly to the site of inflammation, maximizing therapeutic efficacy.

**Reduction of Donor Variability through Pooling and Pooled WJ-MSCs:** One challenge in MSC-based therapies is donor variability, as individual donors often exhibit differences in cell quality and therapeutic potential, leading to inconsistent clinical outcomes. Pooling WJ-MSCs from multiple donors helps reduce this variability by combining cells from different sources, creating a more standardized and reliable product. This approach enhances the consistency of lymphocyte suppression across different batches, leading to more predictable results in treatment. Our findings suggest that pooling significantly improves the reliability of WJ-MSCs, which is crucial for developing effective therapies for SS, where consistent immune modulation is necessary for long-term management.

Additionally, the biological properties of MSCs are influenced by donor-specific factors, resulting in variability in inter-study comparisons. Pooling MSCs from multiple donors mitigates this issue, balancing donor-dependent differences and resulting in a more consistent MSC population. Pooled and cultured WJ-MSCs retain all key MSC characteristics and, based on in vivo toxicity studies, have been shown to be safe while reducing heterogeneity among individual donors. Compared to MSCs derived from a single donor, pooled WJ-MSCs may exhibit enhanced immunosuppressive properties due to the broader spectrum of immune-modulating factors contributed by multiple donors [63]. Investigating the direct injection of pooled WJ-MSCs into the salivary glands of SS patients and evaluating their potential to improve salivary flow rate would be of great interest in furthering the development of MSC-based therapies for SS.

### 1.11 Rationale of the Study

The exploration of treatments for oral complications associated with Sjögren's Syndrome remains insufficiently addressed in current literature. WJ-MSCs are well-known for their anti-inflammatory properties, which are expected to significantly improve the quality of life for individuals with SS by targeting inflammation in the salivary glands. The non-surgical, intraglandular administration of WJ-MSCs presents a promising alternative for treating this condition, enabling precise delivery to the affected tissues. This study aims to evaluate the efficacy of WJ-MSCs in enhancing saliva production through intraglandular injection as a therapeutic approach for SS. Additionally, the study investigates the impact of submucosal (SM) injection on restoring salivary gland function in NOD mice. Histopathological examination of serial H&E-stained sections from the salivary glands will be performed to assess lymphocytic infiltration levels. Furthermore, blood glucose levels in NOD mice will be continuously monitored to explore potential associations between the treatment and metabolic changes.

The aims of this study are as follows:

I. Measure the Salivary Flow Rate (SFR) in NOD mice across all experimental groups, both treated and untreated with WJ-MSCs, as a surrogate marker for salivary gland function.
II. Monitor the effect of WJ-MSC administration on the onset of diabetes by continuously measuring blood glucose levels in NOD mice aged 11 to 16 weeks across all experimental groups.
III. Assess the extent of lymphocytic infiltration in the salivary glands of NOD mice across all experimental groups by analyzing histopathological features, employing lymphocytic

focus scores, and comparing infiltration levels to investigate potential correlations between SS and SG inflammation.

## 2. Materials and Methods

### 2.1 Animal model

All experimental procedures adhered to the guidelines established by the Canadian Council on Animal Care. The protocol for this study (5330) received approval from the University Animal Care Committee (UACC), ensuring compliance with McGill ethical standards and regulations for animal research.

### 2.2 Recipient

Eight-week-old female NOD mice were procured from Taconic Farms (Germantown, NY, USA) and randomized into two distinct groups: the experimental cohort treated with WJ-MSCs (n = 8) and the control cohort receiving normal saline (n = 7).

### 2.3 Blood Glucose Monitoring

Starting at 11 weeks of age, NOD mice underwent weekly evaluations of salivary secretion to assess glandular function, using Schirmer's test strips under inhalation isoflurane anesthesia to ensure consistent measurements ([86], [87]). Mice exhibiting a marked reduction in salivary output (less than 0.1 mL/min) were placed on an intensive monitoring schedule, which included supplemental hydration and daily health assessments. Those showing moderate decreases in flow (0.1–0.2 mL/min) were monitored twice weekly to identify any further decline or the need for additional care. We did not measure water consumption, and it remains unclear whether our animal

facility supports this capability. Mice maintaining typical salivary flow rates continued a weekly monitoring schedule, with increased vigilance if any signs of reduced secretion appeared.

### 2.4 Wharton Jelly Mesenchymal Stromal Cells (WJ-MSCs)

All experimental procedures and handling of MSCs were conducted in strict accordance with the established Standard Operating Procedures (SOPs) of the Cellular Therapy Laboratory (CTL) at the Research Institute of McGill University Health Centre. These SOPs ensure the highest standards of quality, safety, and compliance with regulatory guidelines.

#### 2.4.1 Isolation of WJ-MSC

WJ-MSCs collected from umbilical cords, which is typically discarded after childbirth. The collection of WJ-MSCs is a relatively simple, non-invasive procedure that does not harm the donor or require anesthesia. The collection process involves removing the umbilical cord from the placenta and isolating the Wharton's jelly tissue.

After obtaining informed consent, the umbilical cord (UC) was collected and refrigerated at 4°C. Upon reception, the qualification process involved recording the date and time of qualification, calculating the time lapse between collection and qualification, and measuring the UC's length and diameter (RI-MUHC CTO-SOP R 506). The UC was then weighed without any liquids in the container and segmented into approximately 4 cm sections. The amniotic membrane was incised with a scalpel, and haemostats were used to spread the cord tissue, exposing the Wharton's jelly (WJ). The WJ was then extracted after removal of the arteries and vein and placed into sterile dishes. The extracted WJ was uniformly distributed into 50 mL conical tubes, with a solution containing 500 µg of collagenase, 275 µL of HBSS, 25 µL of Antibiotic-Antimycotic,

and 25 µL of Cord Plasma (CP)/Platelet Lysate (PL) per cm of segment length to ensure soaking of the tissue. The tubes were incubated at 37°C in a water bath for 2 hours, with vigorous mixing every 30 minutes, followed by the addition of a neutralizing solution containing DMEM, Antibiotic-Antimycotic, and CP/PL in twice the volume of the collagenase solution. The mixture was then filtered through a 100 µm Nylon mesh cell strainer. A cell count was performed on the resulting mixture to ensure a minimum concentration of 0.5 x $10^5$/mL, and the cells were plated into coated flasks at 37°C. The media was changed every 2-3 days or until the cells reached 80% confluence.

### 2.4.2 Expansion of WJ-MSC

Upon achieving 80% confluence, but no later than 21 days, the media was aspirated and collected for sterility testing. The flask surface was washed, and Tryple Express solution was used for MSC detachment. After a 10-minute incubation period, the solution was neutralized, and the mixture was centrifuged at 400xg for 10 minutes. The supernatant was removed, and the cells were resuspended in 1 mL of NutriStem mix media. The cells were reconstituted to ensure a concentration of 0.5 ± 0.2 x $10^5$ MSC/mL. Subsequent cell passages were performed as necessary until a maximum concentration of 3 × $10^6$ cells was reached, with a maximum of six passages.

### 2.4.3 Pooling of Expanded WJ-MSC

To create cell pools at a concentration of 1 × $10^6$ cells/mL, cells from each respective donor identification number (DIN), with an initial concentration of approximately 3 × $10^6$ cells, were combined and cultured in coated flasks at 37°C. The media was changed every 2–3 days or when the cells reached 80% confluence. Once the desired confluence was achieved, the cells were

washed, resuspended, and aliquoted into 2 mL vials at a concentration of $1 \times 10^6$ cells/mL. A final concentration of 10% CryoSure-Dex 40 (DMSO)was added to each cryovial, and the vials were stored at -80°C until injection day. To characterize the MSCs, a sample was taken for analysis (RI-MUHC SOP 505)

### 2.4.4 Thawing

On the day of injection, one vial per treatment mouse was thawed in a 37°C water bath until liquefied, which took approximately 2 minutes. After complete thawing, an equal volume of Plasmalyte A with 10% PL was added, and the mixture was cooled in a bucket at 4°C ± 2°C for five minutes. A sample was collected after thawing for a series of analyses to characterize the MSCs.

### 2.4.5 Characterization

WJ-MSCs were characterized to confirm their quality, identity, and functionality. Cell count and viability were evaluated using Bio-Rad TC20 Automated Cell Counter. Additionally, a complete blood count (CBC) was performed on the WJ-MSC preparations to ensure they were free of contaminating blood cells, further validating the purity of the cell population. Additionally, flow cytometry was performed to assess the expression of specific surface markers in accordance with current ISCT recommendations. The WJ-MSCs were positive for CD73, CD90, and CD105, and negative for hematopoietic markers CD34 and CD45, confirming their mesenchymal identity. The multipotency of WJ-MSCs was validated through multilineage differentiation assays, in which the cells were induced to differentiate into adipocytes, osteocytes, chondrocytes, and fibroblasts.

These assays provided functional evidence of the cells' capacity to differentiate into multiple tissue types, thereby confirming their versatility and suitability for potential therapeutic applications.

### 2.5 Injection of Normal Saline and WJ-MSCs

Mice were administered injections at the ages of 9 and 10 weeks. Injections were performed according to the method described by Almansoori et al. [62], in a non-surgical and minimally invasive manner for transcutaneous delivery into the submandibular salivary glands (SGs). Each group of mice received the following treatments: (1) the control group received a single dose of 100 µl of normal saline (NS), 50 µl per SG; (2) the treatment group received a single dose of 100 µl of Wharton's Jelly-derived mesenchymal stem cells (WJ-MSCs), 50 µl per SG, $2x10^6$ WJ-MSCs (a pool of 5). Mice were regularly monitored and sacrificed at 16 weeks of age, at which time serum and SGs were collected for further analysis.

### 2.6 Salivary Flow Rate

Female NOD mice typically experienced a gradual reduction in salivary flow rate around 12-14 weeks of age. To compare the salivary flow rates (SFR) between the control and treatment groups, measurements were taken at various intervals, including 4, 8, and 12 weeks of age. To collect saliva samples, anesthesia was induced with Isoflurane, and 0.5 mg of pilocarpine per kg of body weight was administered subcutaneously to stimulate secretion. During collection, mice were positioned with their heads facing downward to facilitate saliva collection by capillary action, and a heating lamp was used to keep the mice warm throughout the process. Saliva was collected from the oral cavity using a micropipette and placed in pre-weighed 0.5-ml microcentrifuge tubes.

Collection continued for 10 minutes, and the volume of saliva was determined gravimetrically. After saliva collection, the mice were placed on heated plates until fully awake or for 10 minutes.

**(A)** **(B)**

**Figure 2: Procedure for Stimulating and Measuring Salivary Flow Rate in NOD Mice.**

**(A)** Anesthesia induction with Isoflurane, **(B)** 0.5 mg of pilocarpine per kg of body weight administered subcutaneously to stimulate secretion.

### 2.7 Histological Staining and Focus Score

Submandibular salivary gland (SG) tissues were fixed in 4% paraformaldehyde (P6148, Sigma-Aldrich) and subsequently embedded in paraffin. Tissue sections were cut to a thickness of 8 µm and stained with Hematoxylin and Eosin (H&E). These serial H&E-stained histological sections were analyzed to assess the focus score and cytologically examine the area to evaluate the impact of the treatment on the salivary acini. The focus score was quantified as the number of lymphocytic infiltrates per 4 $mm^2$, with a focus defined as an aggregation of approximately 50 lymphocytes. Additionally, the focus area was determined as the area occupied by these lymphocytic infiltrates within the glands, measured in square millimeters.

# 3. Results

## 3.1 Isolation and Expansion of WJ-MSCs Cultures

Throughout the isolation process of WJ-MSCs, the replacement of media at intervals of 7, 10, and 14 days following the isolation procedure prompted the selective attachment of WJ-MSCs to the flask surface, while non-adherent cells were removed. Consequently, all remaining cells exhibited conformity with the criteria for plastic adherence when cultured under standard conditions. Pooled samples also demonstrated adherence to plastic.

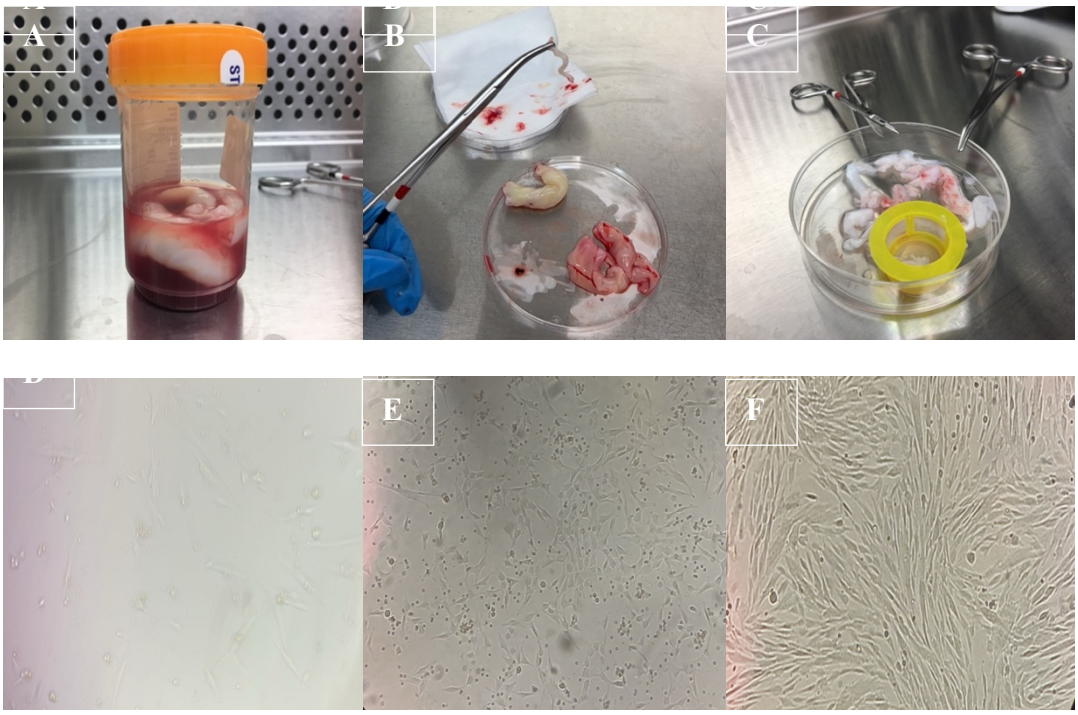



**Figure 3: The sequential stages involved in the isolation and expansion of Wharton's Jelly-derived mesenchymal stem cells (WJ-MSCs).** Panel A depicts the umbilical cord immersed in PBS-EDTA solution immediately after collection. Panel B highlights the vascular structures within the umbilical cord. Panel C shows the Wharton's Jelly region being processed through a cell

strainer. Panels D, E, and F demonstrate the isolated cells at 7-, 10-, and 14-days post-culture, respectively.

### 3.2 Differentiation in WJ-MSCs

**(A)** **(B)**

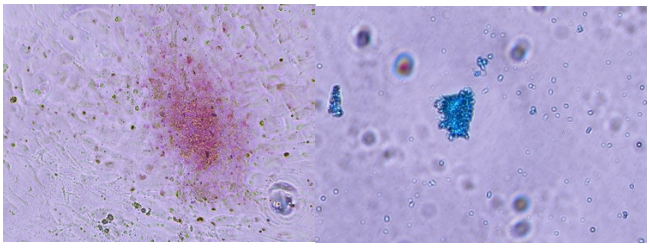
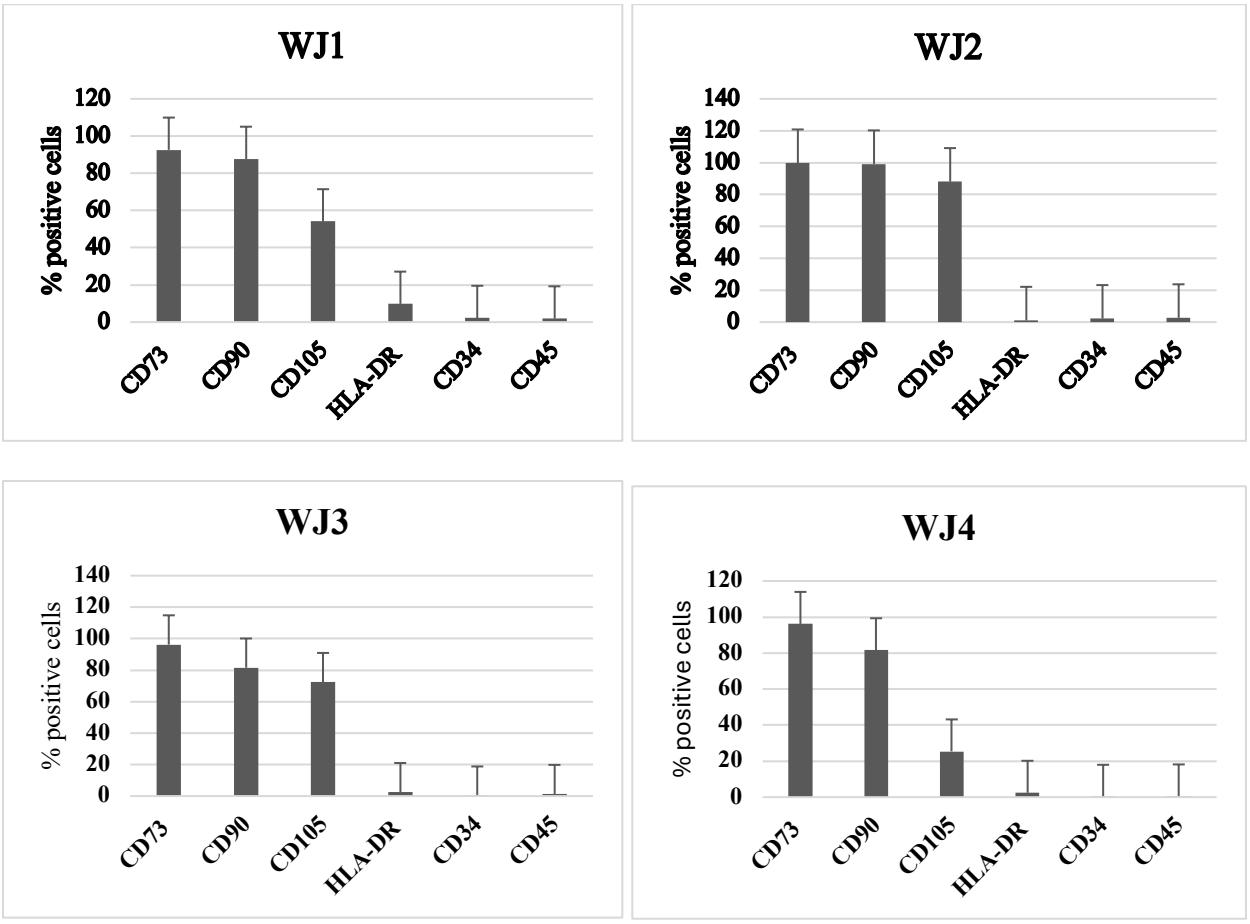


**Figure 4: Pluripotent differentiation capacity of isolated WJ-MSCs into osteoblast-like, adipocyte-like, and chondrocyte-like lineages.** A) Osteocytes, B) Adipocytes, C) Chondrocytes (not shown here)**.** Consistently, a pooled sample comprising the WJ-MSCs from five individual donors exhibited identical results.

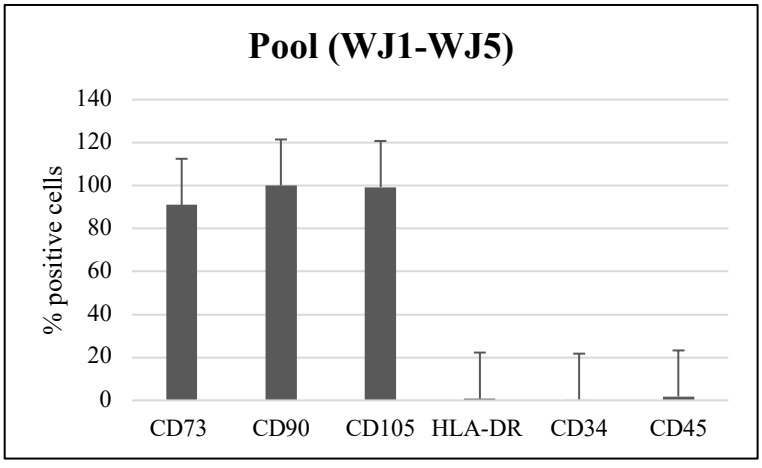
### 3.3 Cell Surface Marker Expression Criteria in Individual Cord Tissue and Pooled sample.

The fundamental prerequisites for WJ-MSCs immunophenotyping entail the presence of CD73, CD90, and CD105 markers, alongside the absence of hematopoietic markers, notably CD34 and CD45. As illustrated in Figure A.

**(A)**


WJ1
% positive cells
120
100
80
60
40
20
0
CD73
CD90
CD105
HLA-DR
CD34
CD45
WJ2
% positive cells
140
120
100
80
60
40
20
0
CD73
CD90
CD105
HLA-DR
CD34
CD45
WJ3
% positive cells
140
120
100
80
60
40
20
0
CD73
CD90
CD105
HLA-DR
CD34
CD45
WJ4
% positive cells
120
100
80
60
40
20
0
CD73
CD90
CD105
HLA-DR
CD34
CD45

**(B)**


Pool (WJ1-WJ5)
% positive cells
140
120
100
80
60
40
20
0
CD73
CD90
CD105
HLA-DR
CD34
CD45

### 3.4 Salivary Gland Functionality

The salivary flow rate (SFR) serves as a fundamental indicator of glandular function, playing a pivotal role in evaluating the effectiveness of therapeutic interventions. Its measurement holds significant value in comprehensively assessing the impact of treatments on salivary gland performance and overall oral health [66]. SFR measurements were obtained at three consecutive time points: prior to treatment at week 0 (at 8 weeks old), and subsequently at 4- and 8-weeks post-treatment. Examination of SFR indicated that untreated control NOD mice consistently exhibited low SFR levels throughout the study duration, reaching their nadir at 4 weeks post-treatment (12 weeks old). In contrast, the groups treated with WJ-MSC displayed elevated SFR values compared to the control group at 4- and 6-weeks post-treatment. Statistical analysis demonstrated that SFR levels in the WJ-MSC treatment groups were significantly higher at 8 weeks post-treatment compared to the control group, with a p-value $< 0.05$.

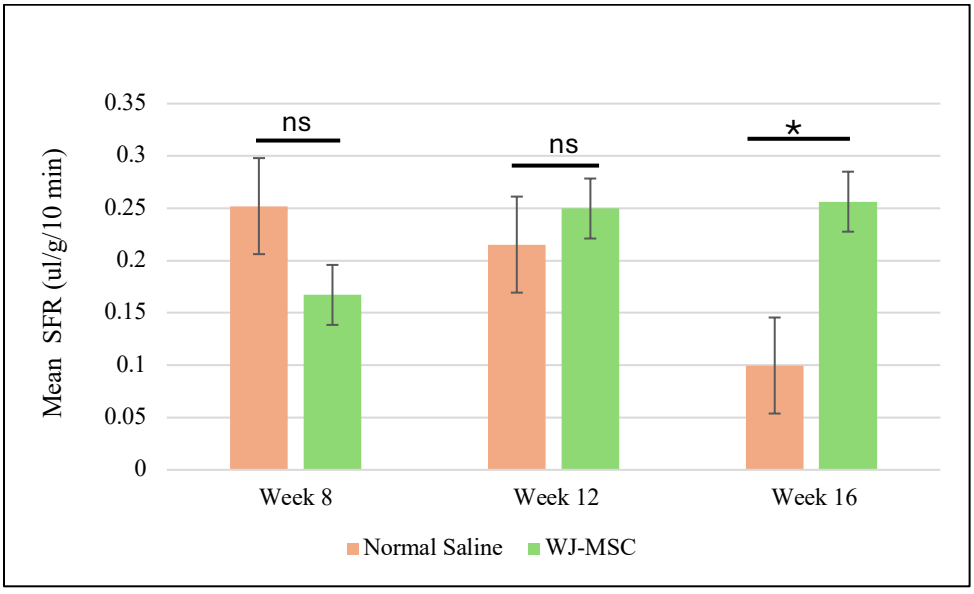



**Figure 5: Variations in Salivary Flow Rate (SFR).** Measurements of SFR were conducted prior to treatment at week 0 (when the mice were 8 weeks old), and subsequently at 4- and 8-weeks

following treatment. The SFR was determined by measuring the volume of saliva produced/gram of body weight over a 10-minute interval. Throughout the study, the control group maintained a consistently low SFR. Results showed that at $12^{th}$ and $16^{th}$ weeks. WJ-MSCs group demonstrated an increase in mean SFR when compared to NS group. * $p < 0.05$. All data were presented as mean ± S.D.

### 3.5 Glucose Levels

At the beginning of the experiment, all mice were normoglycemic at 8 weeks of age. Kaplan-Meier plot revealed distinct survival trends between the treatment and control groups. Diabetes onset was observed earlier in the treatment group, with the first two diabetic mice diagnosed at 6 weeks post-treatment (14 weeks of age). One of these mice succumbed to diabetes within a week of diagnosis. In contrast, the first diabetic mouse in the control group was diagnosed at 14 weeks of age and similarly succumbed to the condition within a week. Following this initial phase, the survival curves demonstrated differing patterns. In the treatment group, there was no further diabetes onset or mortality after the initial decline at Week 14, suggesting stabilization in disease progression. Conversely, the control group exhibited a gradual decline in survival, with additional diabetes cases occurring throughout the observation period. By the end of the 8-week post-treatment observation period (16 weeks of age), most of the treated mice maintained stable glucose levels, while the control group experienced a higher incidence of diabetes. These findings suggest that WJ-MSC treatment has a dual effect on diabetes onset—potentially inducing an earlier but more limited onset, followed by a stabilization phase—whereas the control group experienced continuous diabetes progression. This pattern underscores the importance of examining the temporal dynamics of diabetes onset and associated mortality to better evaluate therapeutic

efficacy. While the exact cause of death in the affected animals cannot be confirmed, the timing suggests a potential link to diabetes progression. Future studies should aim to clarify how WJ-MSC treatment influences both the early onset and subsequent stabilization of diabetes.

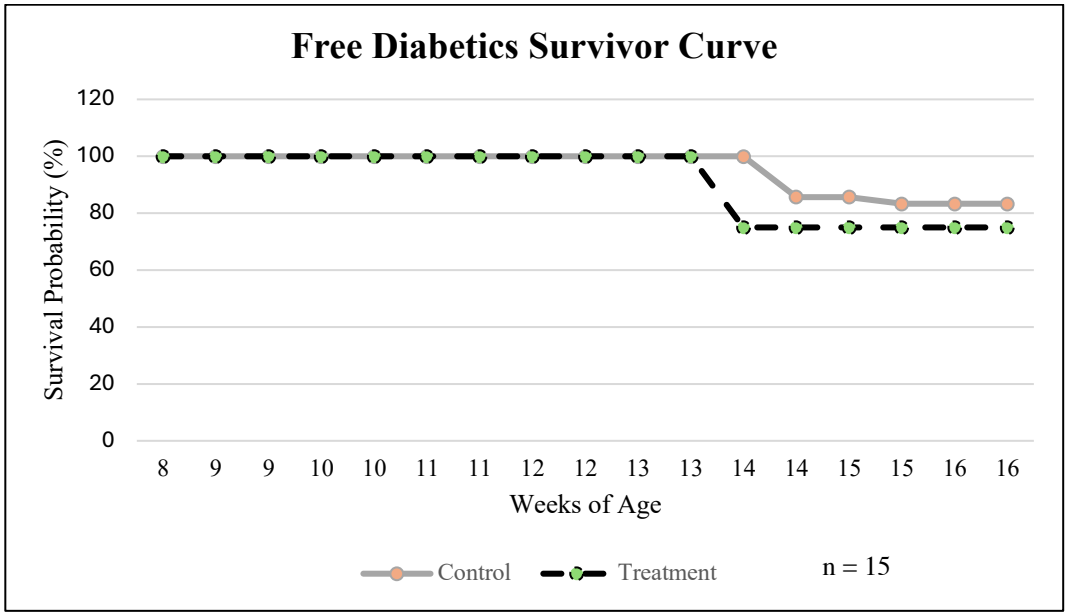


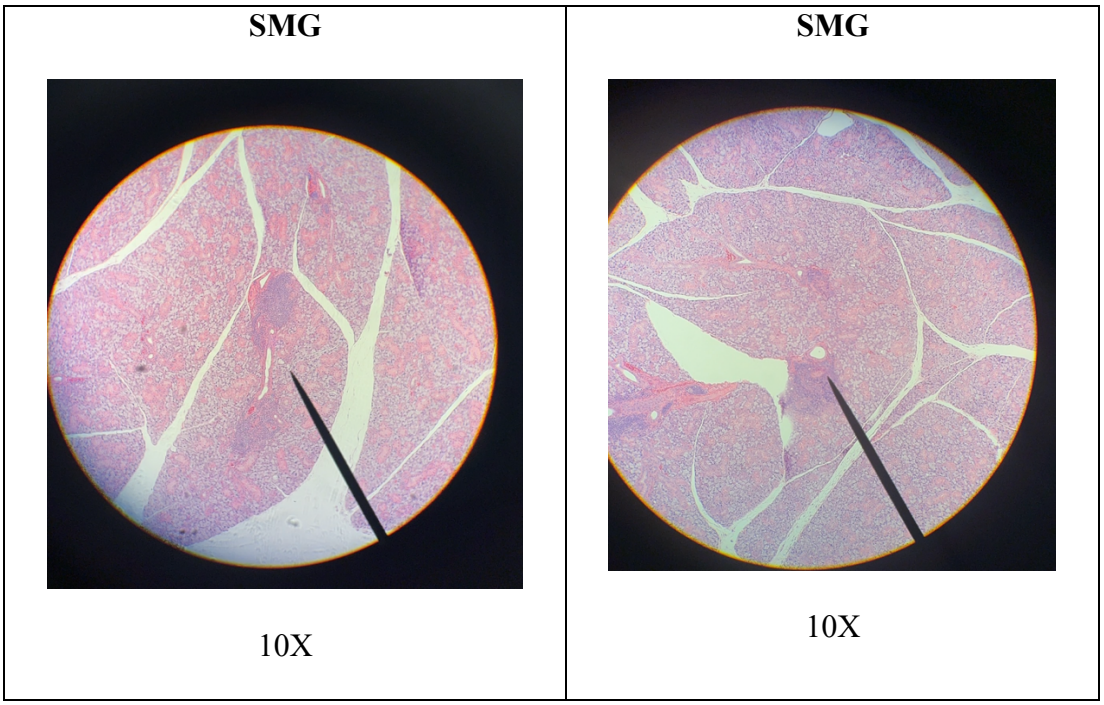

**Figure 6: Diabetes Onset.** All mice were normoglycemic at the start of the experiment (week 0, 8 weeks of age). The first instances of diabetes were observed in the treatment group, with two mice diagnosed at 6 weeks post-treatment (14 weeks of age). One of these mice died within a week of diagnosis. In the control group, the first diabetic mouse was diagnosed at 14 weeks of age and died within the same week. While these events were temporally associated with diabetes onset, the exact cause of death could not be confirmed.

### 3.6 Immunomodulatory and immunosuppressive effects were observed through a decrease in lymphocytic influx.

The efficacy of treatments for autoimmune diseases relies heavily on their capacity to precisely regulate immune dysregulation, effectively targeting pathogenic cells while simultaneously preserving the integrity of the immune system. This understanding underscores the significance of targeted immunotherapies in addressing immune-mediated inflammatory diseases [67]. In the submandibular and sublingual glands, animals treated with WJ-MSCs demonstrated lower focus scores compared to the control group. The mean focus score was $0.75 \pm 0.38$ for the treatment group versus $1.21 \pm 0.47$ for the control group, with a p-value of 0.0556. While this did not meet the conventional threshold for statistical significance ($p \leq 0.05$), the trend suggests a potential reduction in lymphocytic infiltration following WJ-MSC treatment.

| Control | Treatment |
|---|---|

| SLG | SLG |
|---|---|
|  |  |
| 10X | 10X |




**(B)**

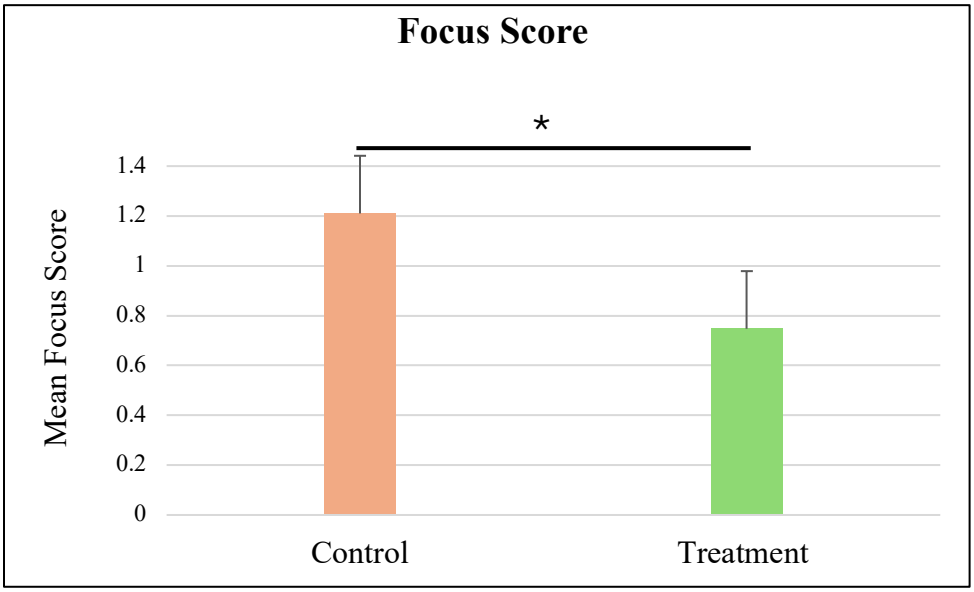



**Figure 7: Focus Score Assessment After an 8-Week Post-Treatment Interval**.

**(A)** The upper panel presents hematoxylin and eosin (H&E) stained images illustrating lymphocytic infiltrates within the sublingual glands (SLGs), whereas the lower panel depicts

similar infiltrates within the submandibular glands (SMGs). **(B)** Focus score quantification of the number of lymphocytic infiltrates per 4 $mm^2$. This analysis was performed on serial H&E-stained sections, which were examined at different depths using light microscopy.

## 4. Discussion

This study presents preliminary evidence on the therapeutic potential of WJ-MSCs in improving salivary gland function, modulating immune responses, and regulating blood glucose levels in an autoimmune model. The multifaceted roles of WJ-MSCs address critical aspects of Sjogren's syndrome and related autoimmune diseases. These findings align with established criteria for the application of mesenchymal stem cells in regenerative medicine, particularly for autoimmune diseases such as Sjogren's syndrome and diabetes.

Control NOD mice consistently exhibited low SFR levels throughout the study, with a notable decline at 12 weeks of age, reflecting the progressive loss of glandular function typical in Sjogren's syndrome. In contrast, mice treated with WJ-MSCs showed a marked improvement in SFR at both 12 and 16 weeks, 8 weeks post-treatment. This finding suggests that WJ-MSCs may play a role in restoring or maintaining salivary function, highlighting their potential as a promising therapeutic option for Sjogren's syndrome. However, further studies with larger sample sizes are needed to confirm these effects, and the relevance to human subjects remains to be evaluated. The sustained increase in SFR in the treatment group suggests a potential role of WJ-MSCs in mitigating glandular damage and improving oral health outcomes in affected individuals.

An important finding of this study is that treatment with WJ-MSCs may influence the progression of diabetes in NOD mice. The Kaplan-Meier analysis revealed that two mice in the treatment group developed diabetes at Week 14, resulting in a sharp decline in survival probability to 75%. However, no additional cases of diabetes were observed in this group, indicating a potential stabilization effect of WJ-MSC treatment after the initial onset. In contrast, the control group exhibited a later onset of diabetes, with the first case occurring at Week 14, followed by a gradual and continuous decline in survival probability, reaching 83.33% by Week 16. This suggests ongoing disease progression in the absence of treatment. While both groups experienced diabetes onset, the data suggest that WJ-MSC treatment may influence the disease trajectory by stabilizing the progression after an initial increase in risk. These results align with the study's objective to assess the efficacy of WJ-MSCs in influencing disease outcomes and suggest that WJ-MSCs may have therapeutic potential in managing diabetes by altering disease progression patterns. This finding contributes to the broader understanding of how cell therapies can impact autoimmune disease progression and management. Further studies are needed to investigate the mechanisms underlying the observed effect and whether it can be sustained over longer observation periods or in different experimental conditions.

An important observation of this study was the decreased lymphocyte infiltration into the salivary glands in the treatment group, indicating that WJ-MSC therapy may have contributed to shielding the glands from additional immune-mediated damage. In SS, the immune system's attack on salivary gland tissue is a central aspect of the disease, causing significant reductions in saliva production. This reduction in immune cell infiltration is crucial, as it likely contributed to the preservation of salivary function, a key factor in managing the symptoms of SS. By limiting the

inflammatory response, WJ-MSC treatment demonstrated an immunomodulatory effect, which plays a pivotal role in preventing the progressive glandular deterioration typically seen in SS patients. These findings emphasize the potential of WJ-MSCs to alleviate autoimmune inflammation, ultimately helping to preserve gland function and improve clinical outcomes in SS.

## 4.1 Limitations and Challenges of Using Wharton's Jelly-Derived Mesenchymal Stromal Cells in Sjögren's Syndrome: Insights from This Study

Although this study highlighted the potential of pooled WJ-MSCs to impact salivary flow rates, it is not without limitations, and several factors require further investigation

**Donor Variability:** Although pooling WJ-MSCs intended to reduce donor-to-donor variability, some variability persisted. Additional standardization steps, such as further refining donor selection criteria or optimizing pooling ratios, *are* necessary to *achieve greater* uniformity in therapeutic outcomes.

**Unclear Long-Term Benefits:** Our research focused on the short-term effects of WJ-MSCs in modulating the immune response and improving glandular function in SS. While the results were promising, with significant improvements in saliva production and lymphocyte suppression, the long-term efficacy of these benefits remains unknown. Given the chronic nature of SS, it is crucial to determine whether these short-term improvements can be sustained over time or if repeated administration of WJ-MSCs will be required. Additionally, there is a lack of data regarding the potential cumulative effects of repeated doses, raising concerns about long-term

safety and therapeutic durability. Future studies should explore extended follow-up periods to evaluate the persistence of these therapeutic effects.

**Determining the Optimal Dosing and Treatment Schedule**: In this study, we used a fixed dose of WJ-MSCs based on previously published protocols; however, the optimal dosage and frequency of administration for SS remain unclear. Our data suggest that while the chosen dose was effective in reducing inflammation and improving gland function, it is uncertain if a higher or lower dose might yield better results or reduce the potential for side effects. Additionally, the timing and frequency of dosing may impact the long-term success of WJ-MSC therapy, especially in a chronic condition like SS. Further research is needed to refine the optimal dosing strategy, including studies that compare different dose levels and treatment schedules to maximize therapeutic benefits while minimizing risks.

**Quality Control Challenges:** While using a single pooled batch of WJ-MSCs in this study minimized variability within the treatment group, producing consistent, high-quality batches for broader clinical use poses significant challenges. In a clinical setting, ensuring uniformity across multiple batches of pooled MSCs is critical. Variations in cell isolation, expansion, and handling across different manufacturing sites could result in inconsistencies in therapeutic efficacy. Even subtle differences in cell viability, growth rates, or immunomodulatory properties could impact treatment outcomes, especially in a condition as complex as Sjögren's syndrome. For WJ-MSC therapy to be viable on a larger scale, stringent quality control measures are required to ensure that each batch maintains consistent potency and therapeutic potential. This includes developing standardized protocols for cell isolation and expansion, as well as rigorous testing to ensure that

all batches meet predefined criteria for purity, functionality, and safety. Variability between batches could lead to unpredictable clinical outcomes, which presents a major challenge for the regulatory approval and widespread adoption of WJ-MSC therapy.

## 5. Conclusion

This study contributes to the expanding body of evidence supporting the therapeutic potential of WJ-MSCs in addressing autoimmune conditions such as SS in NOD female mice. The outcomes presented in this research are promising, that WJ-MSCs may influence key aspects of SS pathology, including the preservation of salivary gland function, modulation of immune responses, and potential regulation of blood glucose levels in an autoimmune model. Notably, the observed improvement in SFR in treated groups, along with reduced lymphocyte infiltration into the salivary glands, provides valuable insights into the mechanisms that may drive the efficacy of WJ-MSC therapy in mitigating glandular damage and maintaining salivary function in SS patients.

Moreover, the WJ-MSC therapy demonstrated safety, ease of administration, and potential scalability for future clinical applications. The ability of WJ-MSCs to modulate immune responses, as reflected the decreased incidence of diabetes and stabilization of survival rates in the treatment group, further emphasizes their broader therapeutic potential in managing autoimmune diseases beyond SS. However, despite these encouraging outcomes, further research is to fully understand the molecular and cellular mechanisms underlying the observed therapeutic benefits. While this study has provided preliminary evidence of WJ-MSC efficacy, more detailed analyses of long-term effects, optimized dosing strategies, and batch consistency are necessary to advance the

clinical translation of this therapy. Additionally, comprehensive safety evaluations are essential to establish the broader applicability of WJ-MSCs in treating SS and related autoimmune diseases.

In conclusion, these findings align with the established role of mesenchymal stem cells in and offer promising avenues for the development of WJ-MSC-based therapies for SS and related autoimmune diseases. Further research is needed to optimize dosing, assess long-term effects, and address quality control and regulatory challenges to unlock the full potential of this therapy and ensure its safety in future clinical applications.

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