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Dental pulp stem cells as a novel antifibrotic therapy for oral submucous fibrosis: An in vitro study

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ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a chronic, potentially malignant disorder associated with areca nut consumption. It is characterized by progressive fibrosis, trismus, and a significant risk of malignancy, with limited treatment options primarily offering symptomatic relief. Dental pulp stem cells (DPSCs), a type of mesenchymal stem cells (MSCs), have shown potential for modulating fibrotic conditions through their immunomodulatory and regenerative properties. This study evaluates the antifibrotic potential of DPSCs on OSMF fibroblasts in an in vitro model.

Methods: DPSCs were isolated from healthy permanent teeth and characterized using flow cytometry for MSC markers (CD73, CD90, CD44, CD105). Fibroblasts were cultured from OSMF biopsy samples and validated through magnetic sorting and morphological analysis. The antifibrotic effects of DPSCs on fibroblasts were evaluated using assays for collagen gel contraction, proliferation, TGF-β1 secretion, and morphological changes. Data were analyzed for statistical significance using appropriate tests.

Results: The mean collagen gel size decreased from 3.235 mm (95 % CI: 1.65–4.82 mm) in the control group to 1.00 mm (95 % CI: −0.27 – 2.27 mm) in the DPSC-treated group. Fibroblast viability declined significantly over 72 h ($p < 0.05$). TGF-β1 secretion was markedly lower in DPSC-treated fibroblasts (339.38 pg/mL vs 637.61 pg/mL, $p = 0.000393$, Cohen's $d = 19.15$).

Conclusion: DPSCs exhibit strong antifibrotic properties by inhibiting collagen contraction, suppressing fibroblast proliferation, and reducing TGF-β1 secretion. These findings suggest DPSCs as a promising cell-based therapy for OSMF. Further in vivo studies are warranted for clinical translation.

Trial registration number: Not applicable.

1. Introduction

Oral submucous fibrosis (OSMF) is a chronic, debilitating, and potentially malignant disorder that primarily affects the Southeast Asian population. It is strongly associated with habitual areca nut chewing, which induces juxta-epithelial inflammation, excessive collagen deposition, and progressive fibrosis of the oral mucosa. This pathological process leads to restricted mouth opening, significant morbidity, and a high risk of malignant transformation (3–9% to oral squamous cell carcinoma).[1,2] The clinical manifestations of OSMF include trismus, oral burning sensations, and stiffness of the oral cavity, which severely impact patients' quality of life.

Despite the growing burden of OSMF, current treatment options, including corticosteroids, hyaluronidase, and physiotherapy, primarily offer symptomatic relief and do not effectively target the underlying fibrotic mechanisms. Given the progressive nature of OSMF and its malignant potential, there is an urgent need for innovative, disease-modifying therapies that can intervene in the fibrotic process and prevent malignant progression.[1–5]

Stem cell-based therapies have gained interest in fibrosis management due to their regenerative and immunomodulatory properties. Mesenchymal stem cells (MSCs), which have been explored in various fibrotic diseases, exhibit anti-inflammatory and antifibrotic effects by modulating cellular signaling pathways involved in extracellular matrix

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(ECM) remodeling.[6,7] Among these, dental pulp stem cells (DPSCs), a type of MSC derived from permanent teeth, offer unique advantages due to their accessibility, multipotency, and regenerative potential. Previous studies have demonstrated that DPSCs can regenerate damaged tissues (bone, dentin, and neural structures) and suppress fibrosis by modulating fibroblast activity.[7–9]

Given the limitations of existing OSMF treatments and the therapeutic potential of DPSCs, this study investigates the antifibrotic effects of DPSCs on fibroblasts cultured from OSMF patients using an in vitro model. The hypothesis is that DPSCs can suppress fibroblast-mediated collagen production, reduce contraction, and modulate key fibrotic pathways, thereby serving as a novel therapeutic approach for OSMF management.[3,4,10,11]

2. Materials and method

Study design: This study was a prospective in vitro experimental study designed to evaluate the antifibrotic effects of dental pulp stem cells (DPSCs) derived from permanent teeth on fibroblasts cultured from oral submucous fibrosis (OSMF) patients. The research was conducted following ethical guidelines and was approved by the Institutional Ethics Committee (IEC) KAHER ETHICS COMMITTE, with approval number KAHER/EC/21–22/D-290721002/2.

Sample size:

Biopsy samples from clinically diagnosed OSMF patients were used to culture fibroblasts (n = 6).

DPSCs were isolated from healthy permanent teeth extracted for orthodontic purposes. Methodology: The study was done in three phases.

2.1. Phase I:Isolation of dental pulp stem cells (DPSCs)

Healthy permanent tooth, indicated for orthodontic extraction, were obtained from individuals aged 16–45 years who were free from systemic diseases and harmful oral habits, such as tobacco and areca nut use. Teeth with carious lesions, periapical or periodontal infections, fractures, or severe attrition were excluded from the study. Following the administration of the local anesthesia, the tooth were extracted under aseptic conditions. After extraction, the teeth were disinfected by immersing in the 3 % sodium hypochlorite solution for 2 min, followed by thorough rinsing with saline. The crowns were sectioned at CEJ using diamond cutting bur to expose the dental pulp, which was extirpated and placed in falcon tube containing fetal bovine serum(FBS) for transportation to the laboratory for cell culture.[12–14]

In the laboratory, the extracted pulp was thoroughly washed in phosphate buffered saline(PBS) containing 1 % pencillin streptomycin. The tissue was then aseptically minced into small pieces(1–2 mm) followed by PBS wash. The minced tissue was placed in 35 mm culture dishes containing alpha modified eagle's medium (α-MEM) supplemented with 20%FBS,100U/ml penicillin, and 100 μg/ml streptomycin. The cultures were maintained at 37 °C with 5 % CO_2 and 80–90 % humidity. Once cells reached 90 % confluence, they were trypsinized using 0.025 % trypsin EDTA solution. The cells were then washed with Dulbecco's Phosphate Buffered Saline(D-PBS) and seeded into T25 flasks for further expansion under same culture conditions.[12–14]

2.1.1. Characterization of DPSCs

Once cells reached 80–90 % confluence, the DPSCs were trypsinized and subcultured into fresh T25 flasks. Flow cytometry was used to characterize the cells, confirming the expression of mesenchymal stem cell (MSc) specific markers. The result demonstrated that high percentage of cells expressed CD73 (99.17 %), CD90 (98.12 %), CD44 (97.91 %), and CD105 (98.91 %), which are indicative of MSCs. These finding were further supported by STR profiling which validated the human origin of DPSCs without contamination.

2.2. Phase II:Culturing of oral submucous fibrosis (OSMF) fibroblasts

Biopsy samples were taken from six patients aged between 20 and 50 years, all diagnosed with Grade II,III or IVa OSMF. Patients with IVb OSMF or those with other precancerous conditions or lesions were excluded from the study. The biopsy samples were approximately 0.5 cm × 0.5 cm x 0.5 cm in size, and were obtained from the fibrotic area of buccal mucosa under local anesthesia. They were transported in Dulbecco's eagle medium(DMEM) to the pathophysiology lab for diagnostic confirmation.

The samples were washed with phosphate buffered saline(PBS), minced into small pieces(1–2 mm) and digested using the solution containing 0.1 % trypsin and 0.75 μg/μL collagenase I. The resulting cell suspension was filtered through a 70 μm strainer and fibroblasts were isolated using magnetic activated cell sorting(MACS) with anti-fibroblast Microbeads. The isolated fibroblasts were cultured in alpha MEM supplemented with antibiotics and FBS, and maintained at 37 °C in a humidified atmosphere 5 % CO_2. After 24 h, non-adherent cells were removed to establish a stable culture.

2.2.1. Characterization of OSMF fibroblasts

Once the fibroblasts reached 80–90 % confluence, they were trypnsinized and placed in to fresh T25 flasks. Flow cytometry showed a high expression of (96.52 %), confirming their authenticity. Additionally STR profiling verified the human origin of fibroblasts and ruled out contamination confirming their unique morphological characteristics as fibroblasts from OSMF tissue.

Pretreatment assays: Fibroblasts from OSMF tissue were assessed for proliferation, morphological features using MTS stain and Fibroblast proliferation.

Proliferation Assay: Primary fibroblast cells were cultured in MEM with 10 % FBS and antibiotics at 37 °C in a 5 % CO_2 atmosphere. After trypsinization, cells were resuspended in medium, seeded at 2×10^4 cells per 200 μL, and incubated overnight to observe morphology. Media was refreshed, and cells were incubated for varying durations (0, 24, 48, 72, and 96 h). After incubation, MTT reagent (0.5 mg/mL) was added, and plates were wrapped in foil to protect from light and incubated for 3 h. The MTT reagent was removed, and DMSO was added to dissolve formazan crystals, with absorbance measured at 570 nm using a spectrophotometer or ELISA reader.

Morphological Assessment: Tissue explants were minced into 1–2 mm fragments, placed in 25 cm^2 culture flasks with DMEM supplemented with 10 % fetal bovine serum, penicillin, and streptomycin, and incubated at 37 °C in 5 % CO_2. Approximately 2–3 mL of proliferation media per flask was used to ensure tissue attachment for cell outgrowth. The medium was replaced after observable cell outgrowth.[15,16]

For Masson Trichrome Staining, cells seeded on 12-well plates at 50 % confluency were washed with DPBS and fixed with methanol at 20 °C for 10 min. Cells were stained sequentially with Weigert's hematoxylin (10 min), acid fuchsin solution (5 min), phosphomolybdic acid (5 min), and methyl blue solution (5 min), with washes in distilled water between steps. After treatment with 1 % acetic acid for 3 min cells were dehydrated using alcohol.[15,16](Fig. 1)

TGF-β1 Secretion Assay: Primary fibroblast cells were cultured in MEM with 10 % FBS and antibiotics at 37 °C in a 5 % CO_2 atmosphere. Cells were seeded at 20,000 cells/well in a 96-well plate, incubated for 24 h, and the supernatant collected for TGF-β1 estimation.

Reagent Preparation: All reagents were brought to room temperature. Assay diluent and wash buffers were prepared as per instructions. Serial dilutions of TGF-β1 standards were made, and antibody concentrates were diluted appropriately.

RayBio® Human TGF-beta 1 ELISA Kit, cat number: Catalog #: ELH-TGFb1.

Sample Activation: Cell culture supernatants were activated by acidifying with HCl, incubating for 10 min, and neutralizing with NaOH/HEPES buffer before immediate assay.

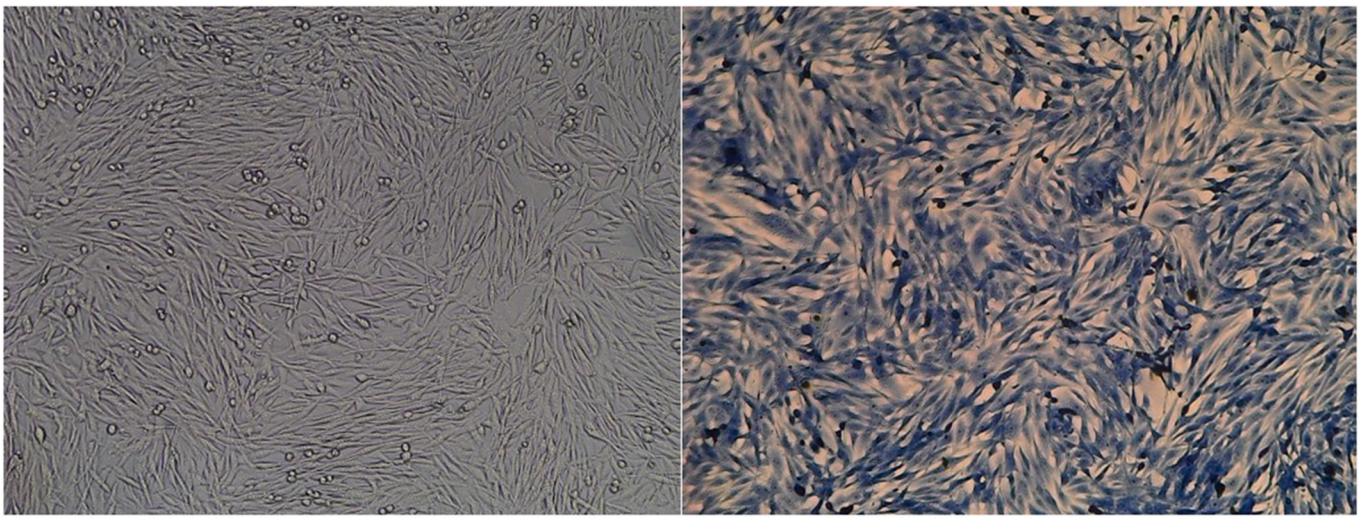

Fig. 1. Morphological assessment of OSMF fibroblasts.

Assay Procedure: Standards and samples were added to a 96-well plate and incubated for 2.5 h at room temperature with gentle shaking. Wells were washed, followed by sequential addition of biotinylated antibody, streptavidin-HRP, and TMB substrate, with washes between each step. The reaction was stopped with a stop solution, and absorbance was measured at 450 nm.

This procedure enabled accurate quantification of TGF-β1 concentrations in cell culture supernatants.[17]

2.3. Phase III: Assessment of antifibrotic effects

In the final phase of the study, the antifibrotic effects of Dental Pulp Stem Cells (DPSCs) on Oral Submucous Fibrosis (OSMF) fibroblasts were evaluated using several experimental methods:

Collagen Gel Contraction Assay: Fibroblast cells (0.5×10^6) were embedded in a collagen gel matrix and cultured for two days. Before initiating the contraction, cells were pretreated with 10 mM BDM(full-form) for 1 h. Changes in gel size(Diameter) over the time were measured to evaluate the impact of DPSCs on collagen fibroblast gel contraction, offering insights into ability of DPSCs to inhibit fibroblasts mediated contraction.[18]

Proliferation Assay: The proliferation rates of OSMF fibroblasts cocultured with DPSCs were measured at 24,48,72 h using cell viability assays. The absorbance readings from these assays provided quantitative data on antiproliferative effects of DPSCs on the fibroblasts, giving a measure of how effectively DPSCs can suppress fibroblast proliferation.

Morphological Assessment: The morphological changes of the fibroblasts cultured with DPSCs were observed using MTS (Masson Trichrome) staining. The intensity of staining along with the any morphologic changes was documented to assess the influence of DPSCs on physical characteristics of fibroblasts demonstrating potential cellular interaction between fibroblasts and stem cells.

TGF-β1 Secretion Assay: The levels of TGF-β1 secreted by the OSMF fibroblasts cocultured with DPSCs were measured using ELISA. The Concentration of TGF-β1 in culture medium was used as a key indicator of antifibrotic effects of DPSCs reflecting their ability to modulate fibroblast activity and reduce fibroblast signaling pathway.

2.4. Statistical analysis

All statistical analyses were performed using SPSS version 25 (IBM, USA). Data were expressed as mean ± standard deviation (SD) unless otherwise stated. The normality of data distribution was assessed using the Shapiro-Wilk test. Based on data distribution, the following statistical tests were used:

1. Collagen Gel Contraction Assay:
   - o Independent *t*-test was used to compare gel contraction between untreated OSMF fibroblasts and DPSCs-treated fibroblasts.
   - o Results are presented with 95 % confidence intervals (CI), p-values, and Cohen's d effect size to determine the strength of the effect.
2. Proliferation Assay (MTT Analysis):
   - o Repeated Measures ANOVA was used to analyze fibroblast viability over 24, 48, and 72 h.
   - o Pairwise comparisons were performed using paired t-tests with Bonferroni correction to adjust for multiple comparisons.
   - o A linear regression model was applied to analyze trends over time.
3. TGF-β1 Secretion Assay:
   - o Independent *t*-test was used to compare TGF-β1 secretion between normal fibroblasts cocultured with DPSCs and OSMF fibroblasts cocultured with DPSCs (Table 6).
   - o Cohen's d effect size was calculated to determine the magnitude of the difference.

   Significance Threshold:
   - o A p-value of < 0.05 was considered statistically significant.
   - o Effect sizes were interpreted based on Cohen's d:
     - Small: 0.2–0.5
     - Medium: 0.5–0.8
     - Large: >0.8

   Graphical Representation:
   - o Error bars in figures represent standard error of the mean (SE).
   - o Figs. 2–4 illustrate statistical differences with *p-value annotations.*

3. Results

3.1. Collagen Gel Contraction Assay

The collagen gel contraction assay demonstrated a significant reduction in contraction in OSMF fibroblasts treated with DPSCs compared to the control group. The mean collagen gel size in the control group was 3.235 mm (95 % CI: 1.65–4.82 mm), whereas in the DPSC-treated group, it significantly reduced to 1.00 mm (95 % CI: −0.27 – 2.27 mm) (Table 1). An independent *t*-test confirmed that this reduction was statistically significant ($p = 0.0185$), with a large effect size (Cohen's $d = 2.59$), indicating a strong antifibrotic impact of DPSCs on fibroblast-mediated collagen contraction (Table 2)(Fig. 2).

Table 1
Effects of DPSCs on collagen gel contraction in OSMF fibroblasts.

Concentration (μM)	OSMF - pretreatment (mm)	OSMF + DPSCs Post treatment (mm)
0	0/0	0/0
50	2.33/2.42	1.1/0.9
100	4.08/4.11	-/-
150	–	–

3.2. Proliferation Assay (MTT analysis)

The proliferation of OSMF fibroblasts was notably diminished over a 72-h period when cocultured with DPSCs. The viability of fibroblasts in the control group at 24, 48, and 72 h was 75 %, 45 %, and 20 %, respectively, showing a clear downward trend over time (Table 3).

Cell viability percentages are shown for OSMF fibroblasts at 24, 48, and 72 h under control and DPSC-treated conditions in Table 3. A significant decrease in fibroblast viability was observed over time in the DPSC coculture group. The extremely low p-value (p = 3.45E-17) confirms that the reduction in viability is highly significant.

Repeated Measures ANOVA confirmed a highly significant reduction in viability ($p < 0.05$), with pairwise t-tests also showing statistically significant differences between:

- 24h vs. 48h (p = 1.33E-05)
- 48h vs. 72h (p = 6.58E-06)
- 24h vs. 72h (p = 2.93E-08) (Table 4)

This decline suggests that DPSCs effectively inhibit fibroblast proliferation, contributing to their antifibrotic potential (Tables 3 and 4) (Fig. 3).

3.3. TGF-β1 Secretion Assay

TGF-β1 levels were markedly higher in OSMF fibroblasts compared to normal oral fibroblasts. However, when OSMF fibroblasts were cocultured with DPSCs, TGF-β1 secretion significantly decreased. The mean TGF-β1 levels in Oral Submucous fibroblasts cocultured with DPSCs were 339.38 ± 5.78 pg/mL, whereas OSMF fibroblasts exhibited significantly higher levels (637.61 ± 10.20 pg/mL) (Table 5). Statistical analysis revealed an extremely significant difference (p = 0.000393) between these groups, with a very large effect size (Cohen's d = 19.15). This suggests that DPSCs strongly modulate TGF-β1 secretion, indicating their potential to mitigate fibrotic signaling in OSMF (Table 6)(Fig. 4).

3.4. Masson Trichrome staining

The Masson Trichrome staining results indicated a marked reduction in extracellular matrix (ECM) deposition in OSMF fibroblasts cocultured with DPSCs. The staining intensity was significantly lower in the treated group compared to controls, suggesting that DPSCs effectively suppress collagen synthesis and deposition. This aligns with the results from the collagen gel contraction and TGF-β1 assays, further supporting their antifibrotic properties(Fig. 5).

4. Discussion

This study provides compelling evidence that dental pulp stem cells

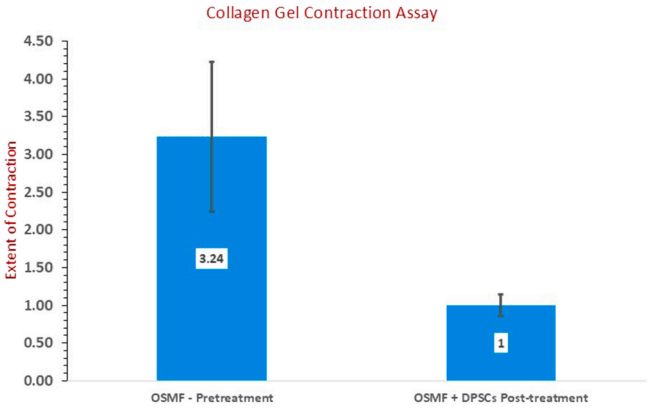

Fig. 2. Collagen gel contraction inhibition by DPSCs.

Table 3
Viability of OSMF fibroblasts cocultured with DPSCs.

Duration of Coculture	Cell Viability (%)	Mean	Std Dev
24 h	75 %	46.66667	27.53785
48 h	45 %	**95 % CI Lower**	**95 % CI Upper**
72 h	20 %	−21.7412	115.0745
F-Statistic	210.2572	P-Value	3.45E-17

Table 4
Pairwise statistical comparisons for fibroblast viability.

Test	Statistic	P-Value
Repeated Measures ANOVA	248.7035	4.05E-18
Paired *t*-test (24h vs 48h)	9.741227	1.33E-05
Paired *t*-test (48h vs 72h)	10.60377	6.58E-06
Paired *t*-test (24h vs 72h)	19.83225	2.93E-08
Linear Regression	−0.00498	0.99665

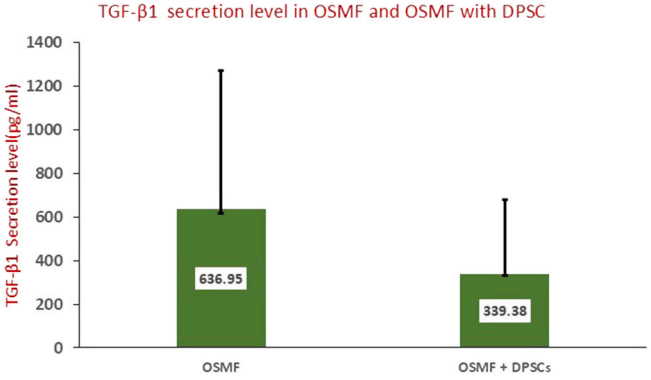

Fig. 3. Proliferation assay of OSMF fibroblasts.

Table 5
TGF-β1 secretion levels in normal oral fibroblasts and OSMF fibroblasts cocultured with DPSCs.

Sample Group	Sample 1	Sample 2	Sample 3	Mean ± SE
OSMF	657.07	616.31	637.46	637.61 ± 10.20 pg/mL
OSMF + DPSCs	330.15	342.07	345.92	339.38 ± 5.78 pg/mL

Table 2
Statistical analysis of collagen gel contraction.

Metric	Mean	Std Dev	95 % CI Lower	95 % CI Upper	T-Test P-Value	Cohenâ€™s d
OSMF - Pretreatment	3.235	0.993797	1.653647	4.816353	0.018496	2.588142
OSMF + DPSCs Post-treatment	1	0.141421	−0.27062	2.27062		

Table 6
Statistical analysis of TGF-β1 secretion levels.

Metric	Mean	Std Dev	95 % CI Lower	95 % CI Upper	T-Test P-Value	Cohenâ€™s d
TGF-β1 OSMF	636.9467	20.38485	586.3079	687.5854		
TGF-β1 (OSMF + DPSCs)	339.38	8.22194	318.9556	359.8044	0.000393	19.14528

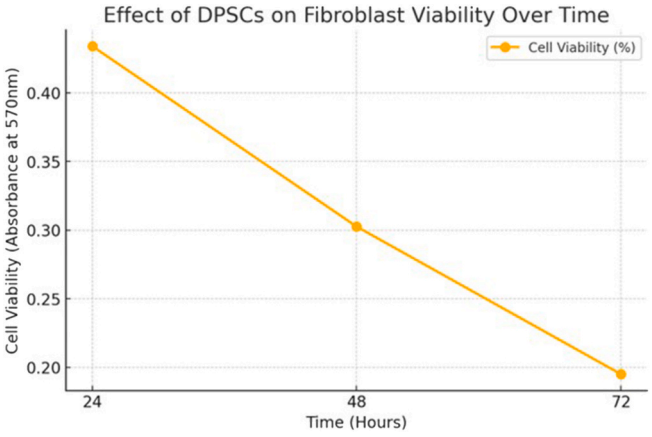

Fig. 4. TGF-β1 secretion analysis via ELISA

(DPSCs) have significant potential as a therapeutic intervention for oral submucous fibrosis (OSMF). OSMF, a chronic and potentially malignant disorder predominantly associated with areca nut consumption, is characterized by progressive fibrosis, restricted mouth opening, and increased risk of malignancy. Despite the availability of conventional therapies, such as corticosteroids and hyaluronidase, these treatments primarily address symptoms and fail to target the underlying fibrotic mechanisms. The antifibrotic effects demonstrated by DPSCs in this study suggest that they may provide a novel, targeted therapeutic approach for this debilitating condition, addressing both fibrosis and its associated complications.

4.1. Inhibition of collagen gel contraction

A primary finding of this study was the significant inhibition of collagen gel contraction in OSMF fibroblasts treated with DPSCs. Collagen contraction is a hallmark of fibrosis, mediated by myofibroblasts exerting contractile forces on the extracellular matrix (ECM). This process is central to fibrotic remodeling and contributes to the dense, nonfunctional tissue seen in OSMF. The observed reduction in collagen contraction suggests that DPSCs disrupt key profibrotic pathways, likely by interfering with myofibroblast activity and reducing ECM deposition.

These findings align with prior studies demonstrating the ability of mesenchymal stem cells (MSCs), including DPSCs, to modulate fibroblast behavior. MSCs exert antifibrotic effects through the secretion of bioactive factors, such as hepatocyte growth factor (HGF) and prostaglandin E2, as well as through direct cell-cell interactions.[7,9] These mechanisms collectively impair myofibroblast function, attenuating collagen deposition and remodeling.[19] Such findings highlight the potential of DPSCs to act as a critical modulator of fibrotic processes, not only in OSMF but potentially in other fibrotic disorders as well.

4.2. Morphological changes and reduced proliferation

This study also observed significant morphological changes and reduced proliferation in OSMF fibroblasts treated with DPSCs. OSMF fibroblasts are characterized by heightened proliferation and collagen synthesis, which contribute to the development of dense, fibrotic tissue. The ability of DPSCs to induce morphological alterations and reduce proliferation underscores their potential to modulate fibroblast behavior and inhibit fibrogenesis.

These effects may be attributed to the paracrine signaling mechanisms of DPSCs. Through the secretion of cytokines such as interleukin-10 (IL-10) and tumor necrosis factor-alpha stimulated gene-6 (TSG-6), DPSCs may reduce fibroblast activation and proliferation.[11]The observed effects on fibroblast morphology and behavior are consistent with the role of MSCs in creating a microenvironment that favors antifibrotic activity and tissue repair, as previously reported in both in vitro and in vivo studies.[10,20]

4.3. Reduction in TGF-β1 secretion

Transforming Growth Factor Beta 1 (TGF-β1) plays a central role in fibrosis by promoting ECM production and driving the differentiation of fibroblasts into myofibroblasts. This study found a significant reduction in TGF-β1 secretion in OSMF fibroblasts cocultured with DPSCs, suggesting that DPSCs target one of the most critical pathways in fibrotic progression. By modulating TGF-β1 signaling, DPSCs may disrupt the molecular cascades that perpetuate fibrosis, leading to a decrease in

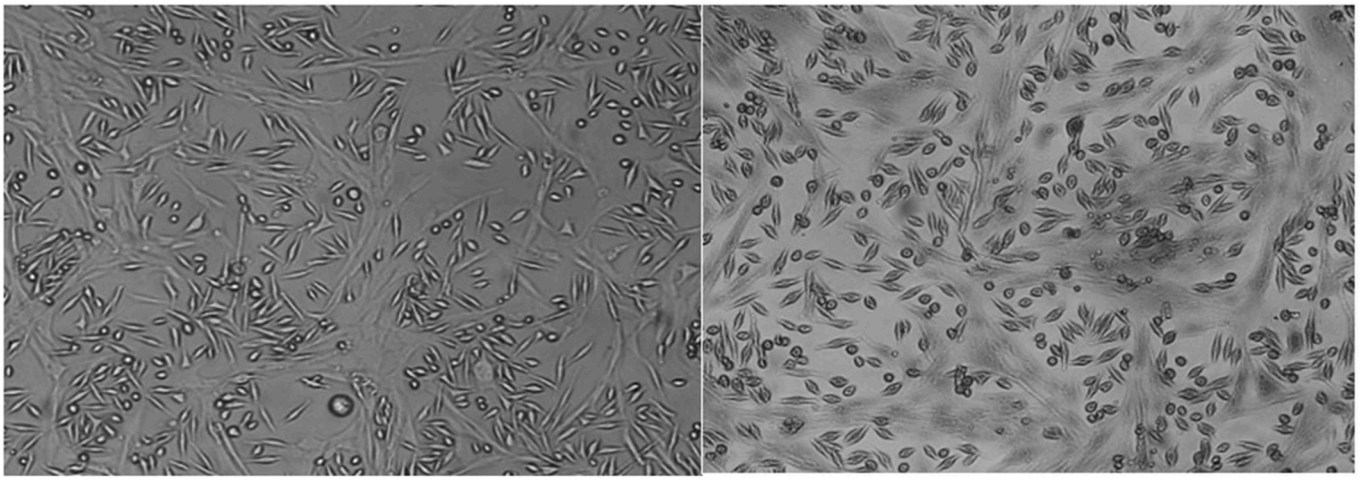

Fig. 5. Morphological changes in OSMF fibroblasts after DPSC coculture.

collagen deposition and fibroblast activation.

The observed reduction in TGF-β1 aligns with previous studies demonstrating the ability of MSCs to suppress profibrotic cytokine activity. For example, Hirata et al. (2016) showed that conditioned medium from stem cells of human exfoliated deciduous teeth (SHEDs) attenuated TGF-β1 activity, resulting in the resolution of fibrosis in a liver fibrosis model. Similarly, Wang et al. (2016) reported that MSCs downregulated TGF-β1 expression in models of immune-mediated diseases, supporting the antifibrotic potential of MSC-based therapies. These findings collectively emphasize the therapeutic relevance of targeting TGF-β1 signaling in fibrotic disorders such as OSMF.[7,10]

4.4. Potential mechanisms of Action

While the precise mechanisms underlying the antifibrotic effects of DPSCs are not fully understood, it is evident that a combination of paracrine signaling and direct cell-cell interactions plays a pivotal role. DPSCs are known to secrete a range of cytokines and growth factors, including HGF, IL-10, and TSG-6, which collectively contribute to their antifibrotic, immunomodulatory, and anti-inflammatory properties.[7,20] These factors have been shown to reduce fibroblast activation, inhibit ECM deposition, and suppress inflammatory responses, creating a microenvironment conducive to tissue repair.

In addition to paracrine effects, direct interactions between DPSCs and fibroblasts may modulate fibroblast behavior. DPSCs express adhesion molecules that facilitate cell-cell contact, enabling them to influence fibroblast activity directly. This interaction likely contributes to the observed inhibition of collagen gel contraction and reduced fibroblast proliferation in this study.

Furthermore, the immunomodulatory properties of DPSCs may play a role in their antifibrotic effects. By modulating macrophage polarization and attenuating inflammatory pathways, DPSCs can reduce the chronic inflammation that drives fibrosis in OSMF.[11,20] The interplay between these mechanisms underscores the multifaceted therapeutic potential of DPSCs in addressing the complex pathology of OSMF.

4.5. Clinical implications

The findings of this study have significant clinical implications. The ability of DPSCs to target key fibrotic processes, including collagen contraction, fibroblast proliferation, and TGF-β1 signaling, positions them as a promising candidate for the development of cell-based therapies for OSMF. Unlike conventional treatments, which primarily address symptoms, DPSC-based therapies have the potential to modify the underlying disease mechanisms, offering a more effective and sustainable solution.

Moreover, the accessibility and multipotency of DPSCs make them an attractive source for regenerative medicine. Their ability to modulate immune responses, reduce inflammation, and promote tissue repair further supports their potential use in a wide range of fibrotic and inflammatory conditions.

5. Conclusion

This study highlights the significant antifibrotic potential of dental pulp stem cells (DPSCs) in oral submucous fibrosis (OSMF). By inhibiting collagen gel contraction, reducing fibroblast proliferation, and modulating TGF-β1 secretion, DPSCs exhibit promising effects on fibroblast activity and extracellular matrix deposition. However, as this research was conducted in vitro, further studies are required to determine the clinical feasibility of using DPSCs in OSMF treatment.

While these findings suggest that DPSCs may hold potential as a therapeutic intervention, it is premature to propose their direct integration into treatment protocols. Future in vivo studies, long-term clinical trials, and mechanistic investigations are necessary to assess their efficacy, safety, and clinical applicability in OSMF management. A comprehensive understanding of their molecular mechanisms and interaction with the oral microenvironment will be crucial in determining their suitability for clinical translation.

5.1. Limitations

While this study provides compelling evidence for the antifibrotic potential of dental pulp stem cells (DPSCs) in oral submucous fibrosis (OSMF), it has several limitations inherent to in vitro models.

1. Absence of Immune System Interactions – The study was conducted in a controlled in vitro environment, which does not fully replicate the complex immune interactions present in vivo. Fibrosis is influenced by inflammatory mediators, immune cell crosstalk, and systemic regulatory factors, all of which were not accounted for in this model. Future studies should incorporate co-culture models with immune cells or animal models to assess the immunomodulatory role of DPSCs in fibrosis regulation.
2. Lack of Oral Microenvironment Dynamics – The in vitro culture conditions do not mimic the physiological conditions of the oral cavity, including vascularization, mechanical forces, and salivary influences, which play critical roles in fibrosis progression and resolution. Future studies should employ 3D organotypic models or animal models to better simulate the oral mucosal environment and evaluate DPSC integration and function in a physiologically relevant setting.
3. Variability in Patient-Derived Fibroblasts – OSMF fibroblasts exhibit heterogeneous behavior depending on disease severity and patient-specific factors. While this study used fibroblasts from six OSMF patients, a larger sample size with fibroblasts at different stages of fibrosis would provide more comprehensive insights into DPSC efficacy across different fibrosis severities.

5.2. Future directions

Building upon these findings, several critical next steps should be explored to facilitate the clinical translation of DPSCs for OSMF treatment:

1. In Vivo Validation – To establish the long-term safety and efficacy of DPSCs in OSMF treatment, preclinical in vivo studies are essential. Animal models can provide insights into cell survival, engraftment efficiency, immune response, and long-term fibrosis modulation in a dynamic oral environment.
2. Optimization of DPSC Delivery Mechanisms – Translating DPSCs into a clinically viable therapy requires the development of efficient and targeted delivery methods. Future studies should explore biomaterial-based scaffolds, injectable hydrogel carriers, or exosome-based therapies to enhance DPSC retention, proliferation, and therapeutic efficacy in fibrotic tissues.
3. Mechanistic Investigations – Although this study demonstrates a reduction in collagen contraction, fibroblast proliferation, and TGF-β1 secretion, the precise molecular mechanisms underlying DPSC-mediated antifibrotic effects remain to be fully elucidated. Future research should focus on analyzing the specific signaling pathways and paracrine factors involved in DPSC-fibroblast interactions, including TGF-β, and PI3K/AKT pathways.
4. Clinical Feasibility and Standardization – Before DPSCs can be considered for clinical application, rigorous clinical trials are needed to establish their safety, efficacy, and reproducibility in OSMF patients. Additionally, standardized DPSC isolation, expansion, and banking protocols should be developed to ensure consistent therapeutic outcomes.

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Self-Funded

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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