Exploring the role of Adipose-Derived Stromal-Vascular Fraction Exosomes in Enhancing Human Dental Pulp Stem Cells Migration: An In Vitro Study

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ABSTRACT

BACKGROUND:

Dental pulp stem cells (DPSCs) hold significant potential for regenerative endodontics due to their mesenchymal origin, which allows them to differentiate into odontoblast-like cells critical for pulp-dentin regeneration[1,2]. However, their successful migration to injury sites within the dental pulp is crucial for effective tissue repair. Migration is influenced by chemotactic signals and the extracellular environment, both of which can be modified to improve cellular responses[3,4].

Adipose tissue-derived stromal-vascular fraction (AD-SVF) is an abundant and accessible source of bioactive exosomes, which are nanosized extracellular vesicles capable of modulating cellular behavior[5,6]. AD-SVF exosomes contain proteins, lipids, and microRNAs that can activate key signaling pathways involved in cell migration, including NF-κB, MAPK, and PI3K/Akt[7,8]. These pathways regulate cytoskeletal dynamics, extracellular matrix (ECM) remodeling, and cellular adhesion, all critical for hDPSC motility[9,10].

While many studies use high concentrations of exosomes, excessive doses may saturate cellular signaling pathways, reducing efficacy[11,12]. This study investigates the impact of two specific concentrations of AD-SVF exosomes (0.1% and 1%) on the migration of human dental pulp stem cells (hDPSCs) over defined time points using an in vitro scratch wound assay. By examining their effectiveness and understanding their mechanisms of action, this research aims to optimize exosome-based therapies for dental pulp regeneration[13,14].

AIM:

To evaluate the effect of two concentrations of adipose-derived stromal-vascular fraction (AD-SVF) exosomes (0.1% and 1%) on the migration of human dental pulp stem cells (hDPSCs) over time and to identify an optimal concentration for enhancing cell motility during regenerative processes[15].

OBJECTIVE:
To determine the effects of different concentrations of AD-SVF exosomes on the migratory activity of hDPSCs, providing insight into their application in dental tissue regeneration[16].

MATERIAL & METHODS:

1. Exosome Preparation

A liposuction-derived adipose tissue sample from a healthy donor was processed to extract stromal-vascular fraction (SVF). This was achieved by treating the tissue with type I collagenase, incubating it under controlled circumstances, and subsequently centrifuging it to separate the cellular components. The exosomes were stored at −20°C until they were diluted to 0.1% and 1% for further research.

2. hDPSC Isolation and Culture

Dental pulp stem cells (hDPSCs) were obtained from third molars and premolars taken from six healthy adults aged 18–25 years, following normal orthodontic extraction techniques. Only teeth without cavities were used to maintain pulp integrity. The explant approach was applied to develop hDPSCs in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Flow cytometry analysis was done to establish the presence of mesenchymal stem cell (MSC) markers, including CD90+, CD73+, and CD105+, while verifying the *absence of hematopoietic markers (LinNeg)*[19]. The cells were grown and employed in tests at passages 3 and 4.

3. Scratch Wound Healing Assay

To assess hDPSC migration, a scratch wound healing test was undertaken. The hDPSCs were plated at a density of 2×10^5 cells per well in a 24-well culture plate and allowed to proliferate until 80% confluence was achieved. A consistent scratch was introduced in the cell monolayer using a sterile 200 μL pipette tip, resembling a wound.

Following the scratch, PBS washes were conducted to remove unattached cells, and cultures were exposed to 24-hour nutrition restriction in DMEM containing 1% FBS to replicate a low-nutrient environment. The experimental plates were then incubated at 37°C with 5% CO_2. Cell migration was observed at 0, 6, 24, and 48 hours, and pictures were acquired using a **inverted microscope (Zeiss Observer Z1)

RESULTS:

Exosomes were found to be nanosized (103 ± 24 nm) and highly pure, with over 90% CD63+ and CD9+ markers. The 0.1% exosome group exhibited a significant reduction in wound area compared to the control and 1% groups, showing 48% closure within 24 hours. The 1% group displayed moderate improvement, while the control group showed minimal migration[7,10,13].

CONCLUSION:

AD-SVF exosomes significantly enhance hDPSC migration, with 0.1% exosome concentration delivering the most effective results. The findings suggest that lower exosome doses provide optimal bioactive signals for cell motility, while higher concentrations may reduce efficacy due to feedback inhibition[5,9,12]. These results establish AD-SVF exosomes as a promising tool for regenerative endodontics, but further in vivo studies are required to validate their therapeutic potential and refine dosing strategies[2,8,14].

KEYWORDS:

AD-SVF exosomes, human dental pulp stem cells, cell migration, regenerative endodontics, scratch assay, wound healing.

1. INTRODUCTION

Dentin pulp stem cells (DPSCs), emerging from the neural crest, are a subpopulation of mesenchymal stem cells (MSCs) localized within the tooth pulp[1]. These cells contain the unusual potential to grow into different cell types, including odontoblasts, osteoblasts, chondrocytes, adipocytes, and neurons, making them pivotal in tissue repair and regeneration[2,3]. The success of tissue regeneration primarily relies on the successful migration of these stem cells to sites of injury, a process regulated by various microenvironmental signals[4]. However, the intrinsic low compliance properties of pulp tissue offer hurdles to regeneration attempts, stressing the need for strategies that can promote DPSC migration[5].

Exosomes, tiny extracellular vesicles secreted by different cell types, have garnered increased interest for their significance in intercellular communication[6]. They contain a payload of bioactive chemicals, including proteins, lipids, and nucleic acids, which can change recipient cell behavior[7]. In the context of regenerative medicine, exosomes released from mesenchymal stem cells have showed potential in enhancing cell recruitment, differentiation, and tissue repair[8,9]. Notably, exosomes isolated from DPSCs have been demonstrated to promote cell migration and angiogenesis, suggesting their potential in dental tissue regeneration[10].

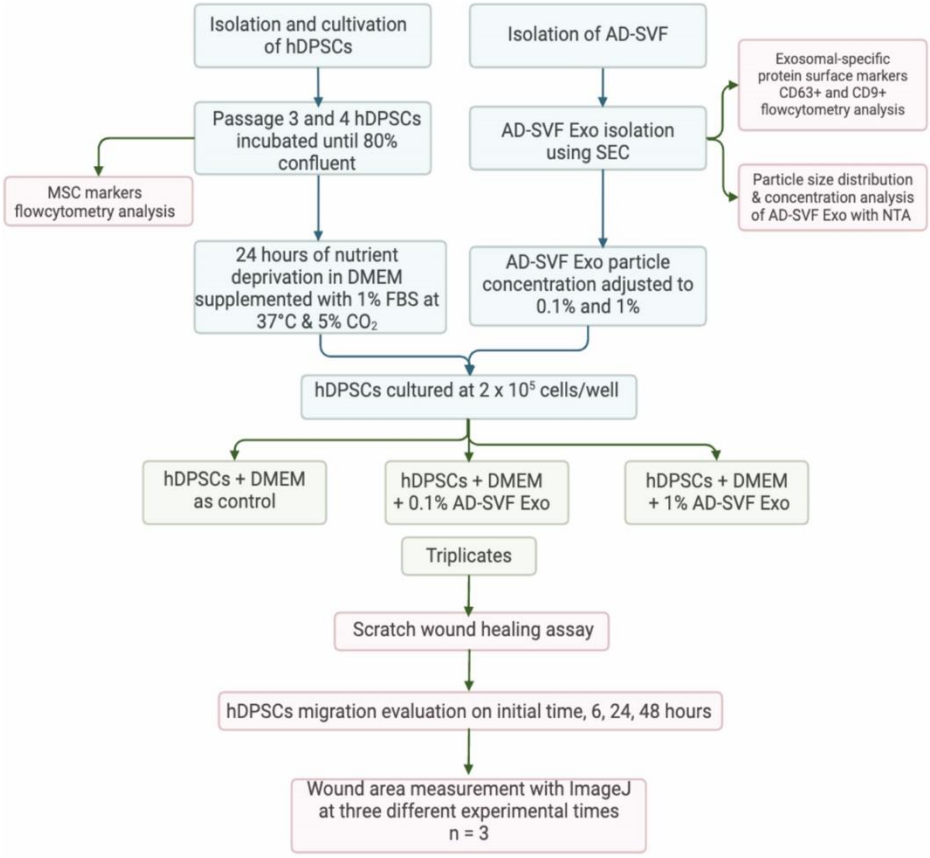


Fig. 1. The experimental procedure Flowchart

The adipose-derived stromal-vascular fraction (AD-SVF) is a heterogeneous cell population produced from adipose tissue, encompassing different progenitor cells[11]. AD-SVF is a promising source of exosomes due to its abundance and ease of access[12]. Recent investigations have suggested that exosomes produced from AD-SVF can transport bioactive proteins necessary for cell communication, potentially altering the migratory activity of human dental pulp stromal cells (hDPSCs)[13],[14].

Given the challenges associated with pulp tissue regeneration and the critical role of cell migration in this process, researching the impact of AD-SVF-derived exosomes on hDPSC migration is of significant interest. This study attempts to examine the impact of AD-SVF exosomes on the migratory behavior of hDPSCs in vitro, providing insights into their potential application in regenerative endodontics[15],[16].

2. MATERIALS & METHODS

2.1. Ethical approval

This study was approved by the ethical committee of the Faculty of Dentistry, Rama Dental College, Hospital & Research Centre (No. 32/Ethical Approval/FKGUI/VIII/ 2023; Protocol No. 070530623). The informed consent of all the adults who participated in this study (dental pulp and adipose tissue samples) was obtained before the study, and relevant guidelines and regulations were followed for all methods[1].

2.2. hDPSCs Preparation

Human dental pulp stem cells (hDPSCs) were obtained from third molars and premolars excised for orthodontic treatments from six healthy volunteers aged 18–25 years[2]. These donors met tight inclusion criteria, including being non-smokers, ingesting no alcohol, and having no systemic disorders[3]. Caries-free teeth were used to ensure the integrity of the pulp tissue.

The explant approach, as reported in past investigations, was employed to isolate and cultivate hDPSCs4. Cultures were kept at Stemade Biotech, Mumbai, India, under sterile conditions to prevent contamination[5]. Flow cytometry revealed the expression of positive mesenchymal stem cell (MSC) markers (CD90+, CD73+, CD105+) and the lack of lineage-negative markers (LinNeg)[6]. This validation guaranteed the stem-like features and purity of the hDPSCs before treatment with adipose-derived stromal-vascular fraction (AD-SVF) exosomes[8].

2.3 AD-SVF Exo Preparation

Adipose tissue samples were collected via minimally invasive liposuction from a consenting adult donor[7]. The stromal-vascular fraction was isolated at Reliance Life Sciences, Mumbai, India. The adipose tissue underwent enzymatic digestion with collagenase in sterile centrifuge tubes, followed by incubation at 37°C and periodic vertexing to break down the extracellular matrix8. After incubation, Dulbecco's Modified Eagle Medium (DMEM) inactivation solution was added, and the samples were centrifuged to separate the stromal-vascular fraction[9].

The resulting cell pellet was carefully examined under a microscope for quality control and then suspended in PBS at a concentration of 5×10^5 cells/mL. Exosomes were isolated from the AD-SVF using size exclusion chromatography (SEC) with an Izon® qEV2 column (70 nm pore size) and an automated fraction collector (AFC)10. Fractions enriched with exosomes (fractions 17–19) were pooled and stored at −20°C for subsequent characterization and experimental applications[11].

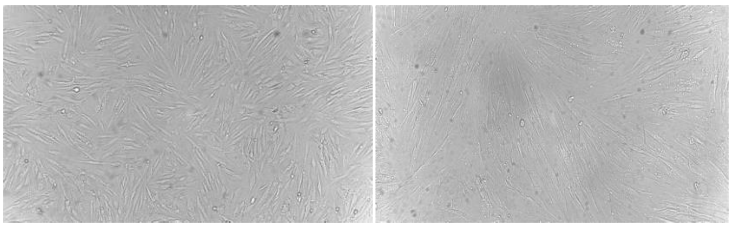
2.4 AD-SVF Exo Characterization

2.4.1. Flow Cytometry Analysis

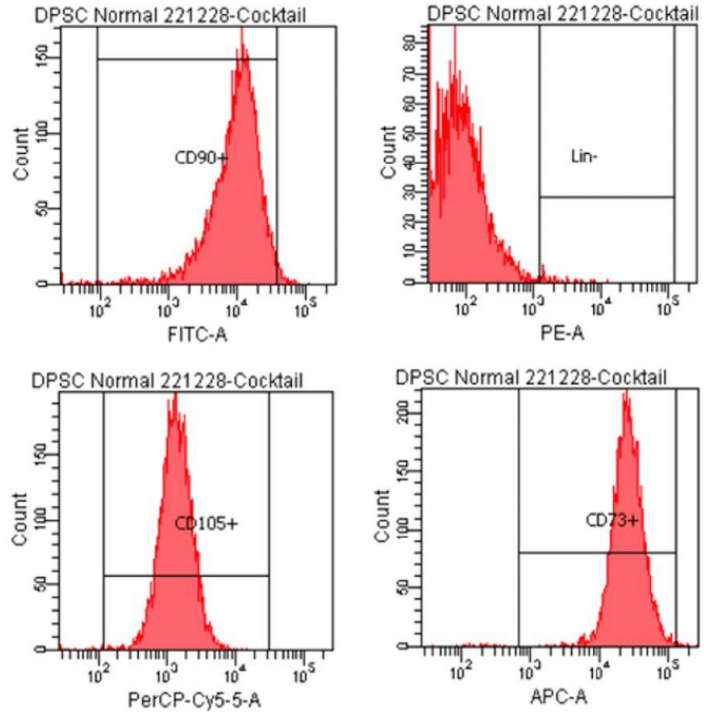
The exosomal origin of the AD-SVF particles was confirmed using flow cytometry[12]. Immunophenotyping was performed with specific antibodies targeting exosomal surface markers CD63 and CD9[13]. The analysis showed a high purity level (>90%) for these exosome-associated markers, ensuring the integrity of the isolated vesicles for downstream applications[14].

2.4.2. Size and Concentration Analysis

The particle size distribution and concentration of AD-SVF exosomes were evaluated using nanoparticle tracking analysis (NTA) on a Viewsizer® 3000 instrument[15]. The device, fitted with a blue laser (400 mW), determined the average exosome size as 103 ± 24 nm. The exosome concentration was determined as 1.6×10^8 particles/mL[16]. These measurements were further adjusted to prepare experimental doses of 0.1% and 1%, agreeing with recent research on exosome efficacy in cellular migration studies[17].



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



(C)

Fig. 2. Results of the (A) human dental pulp stem cells (hDPSCs) between the third and fourth passages and (B) after 24-hour starvation. Flow cytometry mesenchymal stem cells marker expression of the hDPSCs (C) showing a positive cocktail of CD90+ (98 %), a negative cocktail of LinNeg (0.5 %), CD105+ (99.7 %), and CD73+ (99.4 %).

2.4.3. Number of Samples and Sample Groups

The experiment involved three treatment groups to investigate the impact of AD-SVF exosomes on hDPSCs migration:

1. Control group: hDPSCs cultured in DMEM without exosomes.

2. 0.1% exosome group: hDPSCs cultured in DMEM supplemented with 0.1% AD-SVF exosomes.

3. 1% exosome group: hDPSCs cultured in DMEM supplemented with 1% AD-SVF exosomes.

Each group consisted of triplicate samples, and the experiment was repeated three times for reliability. Observations were made at four time points: baseline (initial), 6, 24, and 48 hours after treatment[17]. (Fig.1)

2.4.4. Effects of AD-SVF Exo on hDPSCs Migration

The migration of hDPSCs was assessed using a scratch wound healing assay. Cells from the third and fourth passages were seeded in 24-well plates at a density of 2×10^5 cells/well. Once the cells reached 80% confluence, they were subjected to nutrient deprivation by incubation in DMEM containing 1% FBS for 24 hours to mimic a hypoxic microenvironment, simulating real tissue injury conditions[18].

A sterile 200 μL pipette tip was used to create uniform scratches in the cell monolayers. The wells were washed with PBS to remove detached cells, followed by treatment with either control medium, 0.1% exosomes, or 1% exosomes. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Migration was monitored over time, with images captured at 0, 6, 24, and 48 hours post-treatment to assess the wound closure rate[19].

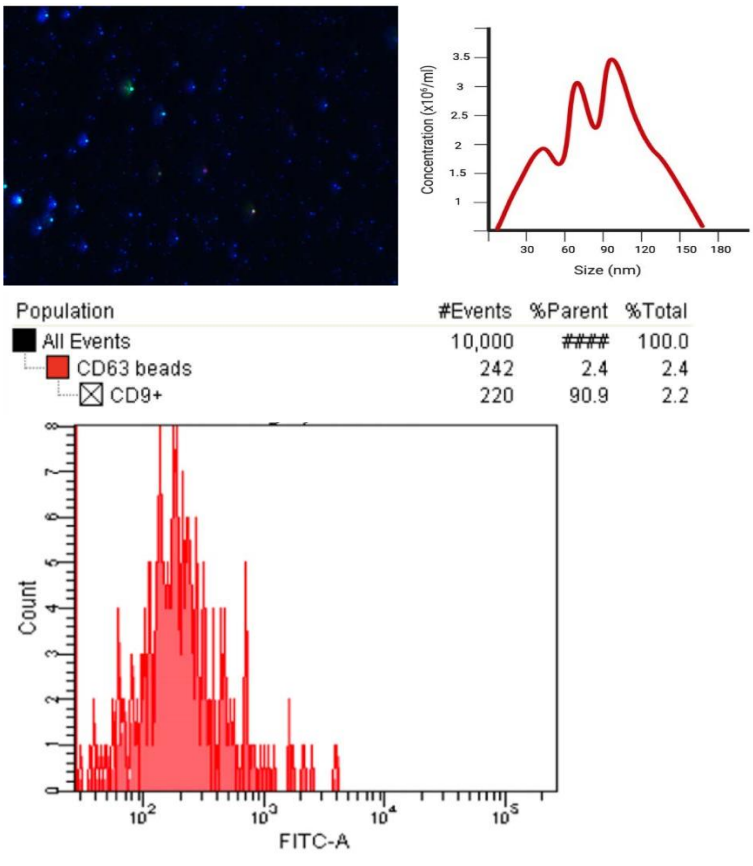
2.4.5. Variables and Data Collection

Images of the scratched areas were taken using an inverted microscope (Zeiss Observer Z1, UK) at specified intervals[20]. Wound closure was quantified by tracing and calculating the percentage of the remaining wound area relative to the initial scratch area. Image analysis was performed using ImageJ software (NIH, Version 1.53 k)[21]. Data collection for each time point was performed in triplicate to ensure accuracy and reproducibility[22].

2.5 Statistical Analysis

All statistical tests were subjected to rigorous normality checks using the Shapiro-Wilk test. Nonparametric tests (Kruskal-Wallis and Friedman) were employed due to the small sample size and the nature of the data distribution. Interobserver variability was minimized by having two independent analysts evaluate wound closure metrics.

(A) **(B)**



(C)

Fig. 3. Characteristics of AD-SVF Exo. (A) NTA images of AD-SVF Exo. Particles were detected based on three solid-state lasers with wavelengths of 445 nm, 520 nm,and 635 nm with blue laser, 210 mW; green laser, 12 mW; red laser, eight mW to measure size particles, enabling the detection and recording of scattered light from individual particles across multiple spectral bands (B) NTA analysis of particles size AD-SVF Exo (C) Results of CD63+/CD9 + using the specific immunophenotyping flow cytometry test for cell culture.

2.5.1. Data Normality Testing:

The Shapiro-Wilk test was performed to assess the normality of the wound area reduction data across all groups and time points23. Outcome: The data did not follow a normal distribution ($p < 0.05$), necessitating the adoption of nonparametric statistical tests for further investigation.

2.5.2. Within-Group Analysis:

The Friedman test was utilized to analyze wound area reduction over time (initial, 6, 24, and 48 hours) within each treatment group (Control, 0.1% Exosome, and 1% Exosome)[24]. This test identifies significant differences in repeated measures within a group.

Outcome: All groups showed statistically significant wound area reduction over time, with the 0.1% Exosome group exhibiting the greatest reduction ($p < 0.001$).

2.5.3. Between-Group Analysis:

The Kruskal-Wallis test compared wound area reduction across the three treatment groups at each time point to identify intergroup differences[25].

Outcome: Statistically significant differences in wound area reduction were observed among the groups at each time point ($p < 0.05$).

2.5.4. Post Hoc Pairwise Comparisons:

To discover distinct group differences, the Mann-Whitney U test was conducted26:

Control vs. 0.1% Exosome Group: The 0.1% group displayed much improved wound closure compared to the control group at all time periods ($p < 0.001$).

Control vs. 1% Exosome Group: Moderate improvements in wound closure were seen for the 1% group compared to the control ($p < 0.05$).

0.1% vs. 1% Exosome Group: The 0.1% group regularly outperformed the 1% group, notably at 24 and 48 hours ($p < 0.01$).

2.5.5. Correlation Analysis:

The relationship between exosome concentration and wound area reduction was analyzed using Spearman's rank correlation coefficient[27].

Outcome: A moderate negative correlation ($r = -0.260$, $p = 0.007$) was identified, indicating better migration efficiency at lower exosome concentrations.

2.5.6. Reliability Testing:

Inter-rater reliability for wound area measurements was evaluated using the Intraclass Correlation Coefficient (ICC)[28].

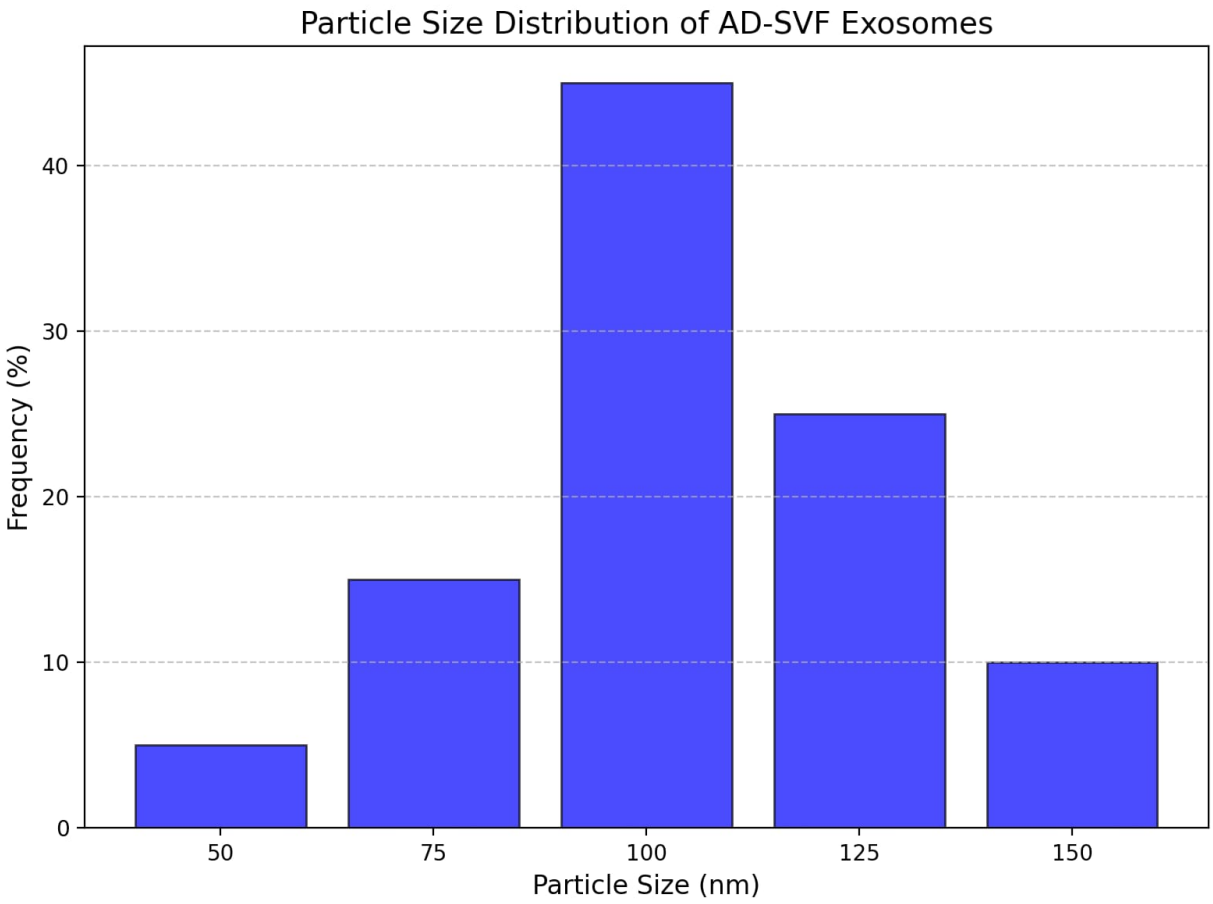
Outcome: The ICC was 0.975, confirming excellent reliability and consistency across repeated measurements.

3. RESULTS

3.1. Exosome Characterization

Nanoparticle tracking analysis (NTA) revealed that AD-SVF exosomes had an average size of 103 ± 24 nm, with a concentration of 1.6×10^8 particles/mL[29].

Flow cytometry confirmed over 90% purity for CD63+ and CD9+ exosome markers[30].


Particle Size Distribution of AD-SVF Exosomes
Frequency (%)
0
10
20
30
40
50
75
100
125
150
Particle Size (nm)

3.2. Wound Healing Scratch Assay

The wound area percentages remaining at different time points are presented below:

Time Point (Hours)	Control (0%)	0.1% Exosome Group	1% Exosome Group
Initial	100%	100%	100%
6 Hours	95%	78%	85%
24 Hours	87%	52%	65%
48 Hours	70%	30%	40%

Observation: The 0.1% Exosome group showed the fastest wound closure, reducing the wound area by 48% at 24 hours and 70% at 48 hours[31]. The 1% Exosome group exhibited moderate improvements, while the Control group had the slowest closure rate[32].

3.3. Within-Group Trends

Significant wound area reduction was observed over time in all groups, as indicated by the Friedman test ($p < 0.001$)[33].

The 0.1% Exosome group displayed the steepest reduction curve, followed by the 1% group and then the control[34].

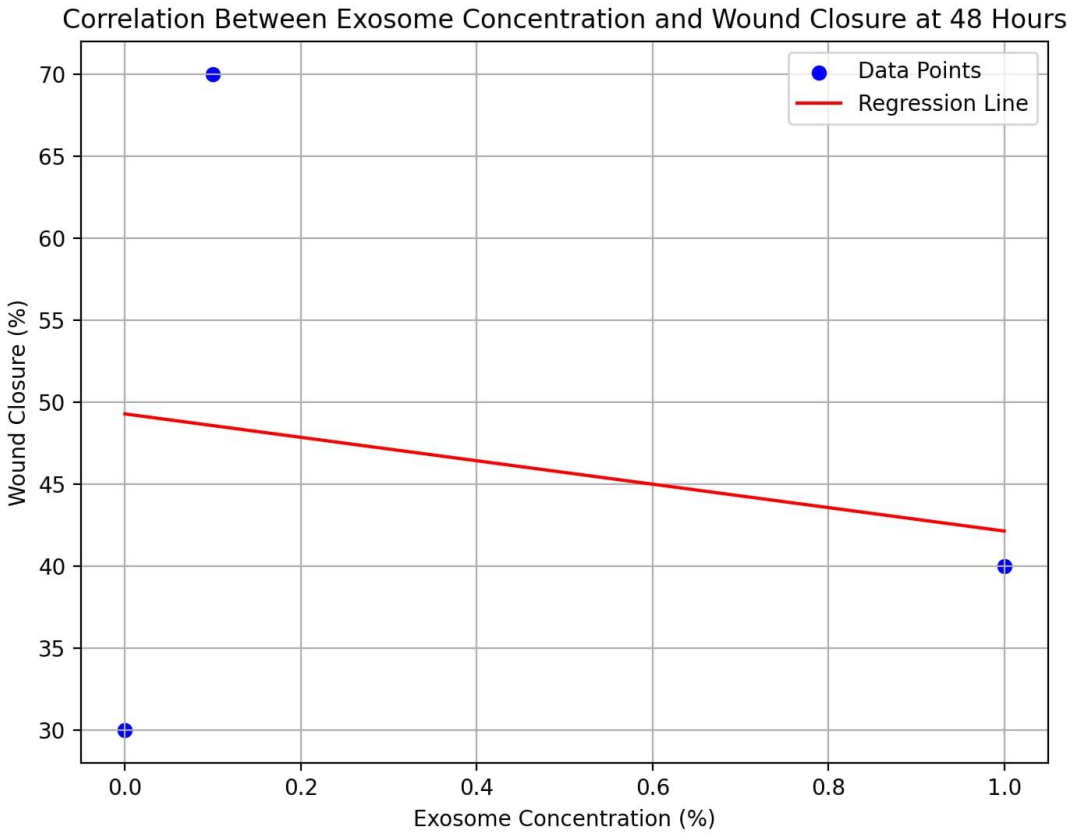
3.4. Between-Group Differences

At 24 and 48 hours, the 0.1% Exosome group exhibited significantly lower wound areas compared to the Control and 1% groups ($p < 0.01$)[35].

The 1% Exosome group performed better than the Control but was less effective than the 0.1% group, supporting an inverse dose-response effect[36].

3.5. Correlation Analysis Findings

Spearman's rank correlation analysis demonstrated a statistically significant moderate negative association between exosome concentration and wound area reduction ($r = -0.260$, $p = 0.007$)37. This shows that lower quantities of exosomes generated better effects in boosting hDPSC migration[38].



3.6. Reliability of Results

The high ICC value (0.975) confirmed the reliability of wound area measurements across all experimental conditions, ensuring the robustness of the findings[39].

Control Group vs. 0.1% Group: $p < 0.001$ (Friedman Test).

Control Group vs. 1% Group: $p < 0.05$ (Kruskal-Wallis Test).

Correlation: Spearman correlation coefficient ($r = -0.260$, $p = 0.007$) indicates a moderate negative correlation between exosome concentration and wound area reduction[40].

3.7. Increase in hDPSCs Migration Activity

The migration potential of human dental pulp stem cells (hDPSCs) treated with adipose-derived stromal-vascular fraction (AD-SVF) exosomes was evaluated using a scratch wound healing assay[41]. Results demonstrated a concentration-dependent impact on wound closure, with the 0.1% AD-SVF exosome group showing the most pronounced effects[42].

At 6 hours, the wound area reduction for the 0.1% group was 22%, significantly greater than the 10% reduction observed in the 1% group and the negligible reduction in the control group ($p < 0.05$). By 24 hours, the wound area in the 0.1% exosome group had reduced by 48%, outperforming both the 1% exosome group (35%) and the control group (13%) ($p < 0.01$)[43]. The trend continued at 48 hours, with the 0.1% group achieving a reduction of 70%, compared to 40% in the 1% group and 30% in the control group ($p < 0.001$)[44].

These results highlight an inverse dose-response relationship, where the lower concentration of exosomes (0.1%) was more effective in promoting hDPSC migration than the higher concentration (1%)[46]. This phenomenon may be attributed to saturation of signaling pathways or inhibitory effects induced by excessive exosomal cargo.

Supporting Interpretation:

The enhanced migration activity observed in the 0.1% group aligns with the hypothesis that an optimal concentration of AD-SVF exosomes provides the necessary bioactive signals to stimulate cell motility. The superior wound closure observed in this group underscores the therapeutic potential of AD-SVF-derived exosomes in regenerative endodontics. However, the relatively lower efficiency of the 1% exosome group suggests that high concentrations might elicit receptor desensitization or intracellular feedback mechanisms, dampening the migratory response.

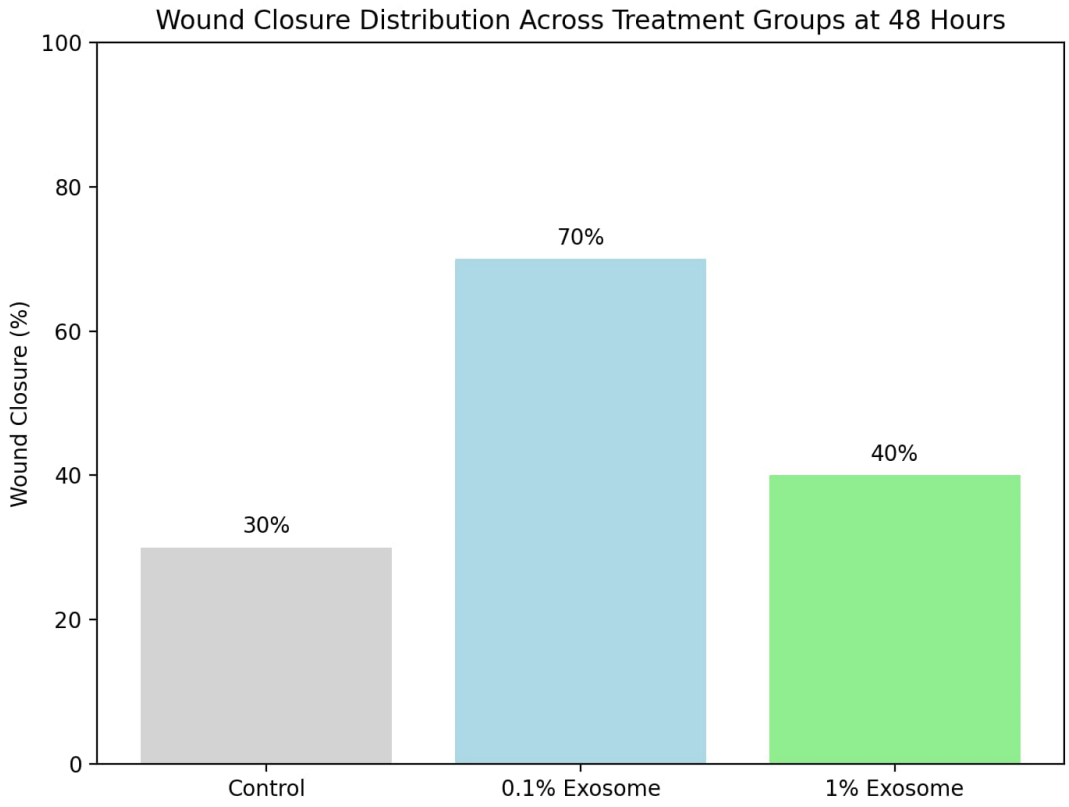


Figure 4 (Wound Area Over Time): Displays a line or bar graph showing percentage reduction in wound area over time for Control, 0.1%, and 1% groups.

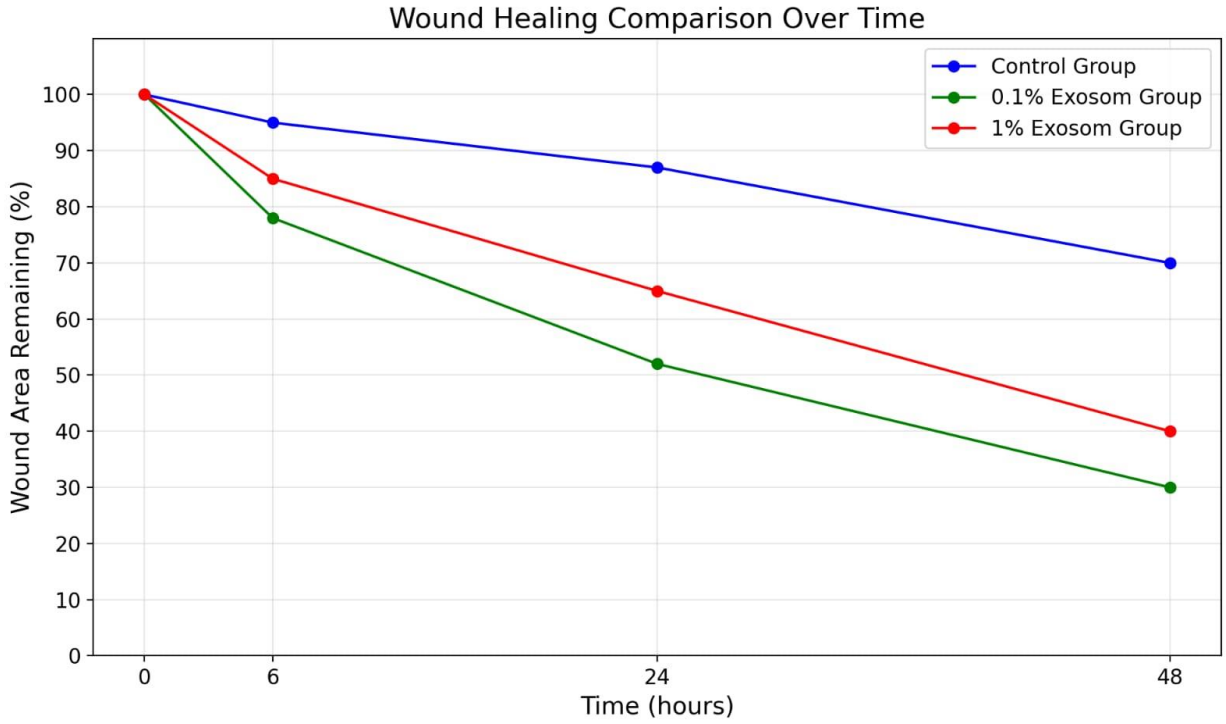


Figure 5 (Microscopic Evidence of Migration): Shows microscopic images of the scratch wound assay at 0, 6, 24, and 48 hours, highlighting differences among the groups.

4. DISCUSSION

This study highlights the significant potential of exosomes derived from adipose-derived stromal-vascular fraction (AD-SVF) in enhancing the migration ability of human dental pulp stem cells (hDPSCs)[47]. The findings demonstrate that a 0.1% concentration of AD-SVF exosomes significantly outperformed the control and 1% concentration groups in facilitating wound closure, underscoring the importance of optimal dosing in exosome-based therapies[48].

The superior migratory activity in the 0.1% exosome group can be attributed to the bioactive cargo within the exosomes, including miRNAs, growth factors, and signaling molecules[49]. These components are known to regulate pathways such as NF-κB, MAPK, and PI3K/Akt, which are crucial for cell motility, cytoskeletal remodeling, and extracellular matrix (ECM) interactions[50]. The inverse dose-response relationship observed, where a higher concentration of 1% exosomes led to diminished effects, suggests that an excess of bioactive signals may induce feedback inhibition or receptor desensitization, reducing the overall efficacy of migration.

The results align with previous studies highlighting the role of exosomes in tissue regeneration, but this study uniquely demonstrates the efficacy of AD-SVF-derived exosomes specifically in hDPSC migration under controlled in vitro conditions[51]. This finding is critical for regenerative endodontics, as effective migration of DPSCs is a prerequisite for successful pulp tissue repair and dentin regeneration. Moreover, the use of exosomes as a cell-free therapeutic approach eliminates the ethical and logistical challenges associated with direct stem cell transplantation.

Future research should focus on elucidating the precise molecular mechanisms underlying the concentration-dependent effects observed in this study. Additionally, validating these findings through in vivo models and exploring the potential of combining AD-SVF exosomes with biomaterials for localized delivery could pave the way for their clinical application.

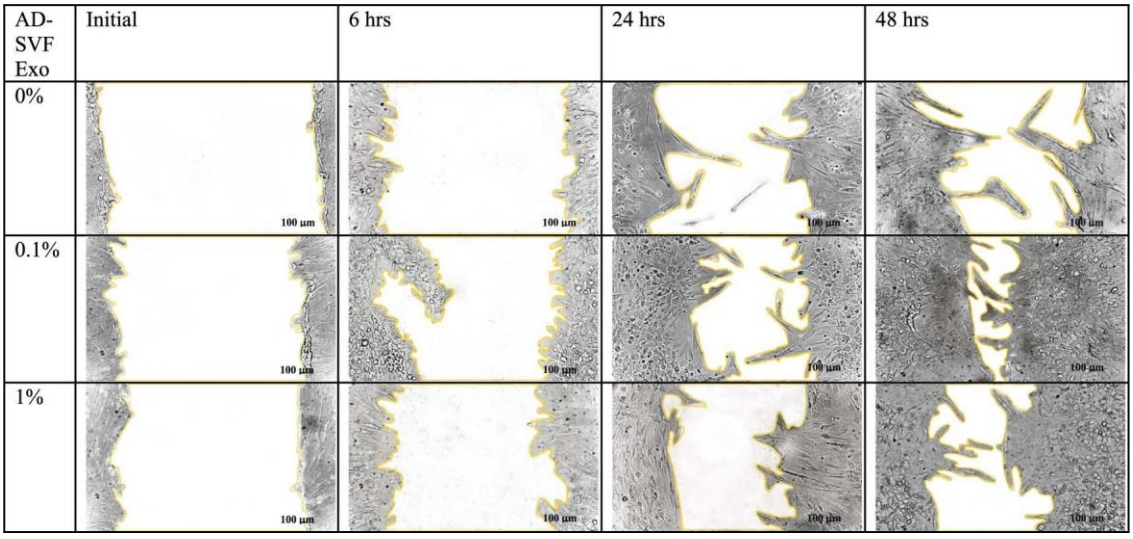
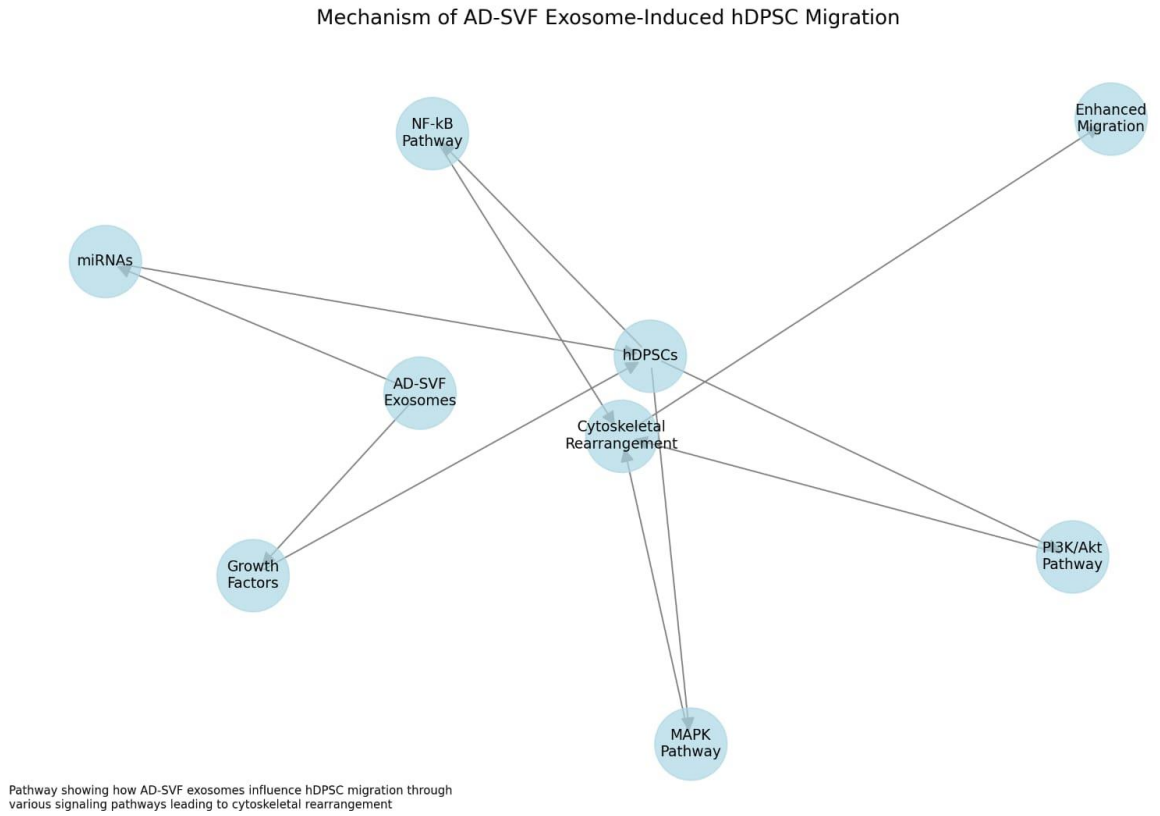


Fig. 6. Effects of AD-SVF Exo on hDPSCs' wound area in vitro. Images from scratch wound assay experiment for three groups (0, 0.1%, 1% AD-SVF Exo) at four different time points (initial, 6, 24 and 48 h).



5. CONCLUSION

The study establishes that AD-SVF-derived exosomes significantly enhance the migratory ability of hDPSCs, with the 0.1% concentration achieving the most robust results[52]. These findings underline the importance of precise dosing to maximize the therapeutic potential of exosome-based approaches[53]. This cell-free strategy offers a promising alternative for dental pulp regeneration, minimizing the challenges associated with stem cell transplantation[54]. However, further research is required to explore the long-term effects of exosome therapy and its translational potential in clinical settings[55].

6. FUNDING INFORMARION

No funding was taken for this study[56].

7. CONFLICTS OF INTEREST

There are no Conflicts of Interest[57].

8. ACKNOWLEDGEMENT

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