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Research Article

Differential Effects of Bone Morphogenic Protein (BMP) and Vascular Endothelial Growth Factor (VEGF) on Dental Pulp Stem Cell (DPSC) Subpopulations

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Abstract

Introduction: Recent work has demonstrated therapeutic and clinical applications for autologous dental pulp stem cells (DPSC) for bone tissue regeneration and bioengineering. Bone morphogenic protein (BMP) is a powerful growth factor that may be sufficient to modulate and regulate Mesenchymal Stem Cell (MSC) (and potentially DPSC) differentiation. Therefore, the primary objective of the current study was to evaluate the potential for BMP (alone and in combination) to induce changes to specific DPSC phenotypes.

Methods: Using previously isolated DPSC, six (n = 6) isolates were treated with either BMP-2, Vascular Endothelial Growth Factor (VEGF) or both. Trypan blue viability assays and 96-well growth and proliferation assays were performed to determine any changes to DPSC phenotypes.

Results: The data clearly demonstrated that one subset of DPSC isolates (slow doubling time) exhibited significant increased viability under both BMP-2 and combined VEGF-BMP-2 administration. Differential and variable increases in cell growth were observed among the corresponding slow DPSC isolates, which suggests more complex relationships between growth and growth factor administration may be present. No similar effects were observed with the other subset of DPSC isolates (intermediate doubling time) and no effects on either growth or viability were observed under VEGF administration alone.

Conclusion: Although only a limited number of DSPC isolates could be evaluated, the data from this study revealed a strong and significant change to sDT DPSC phenotypes (growth and viability) under BMP-2 administration (either alone or in combination with VEGF). These changes appear to be restricted to this subpopulation of DPSC, which may provide some insight into the mechanisms and underlying biology responsible for these observations. Future studies may be needed to evaluate the potential for BMP-2 and other growth factors to induce DPSC differentiation and lineage-specific phenotypic changes for bioengineering applications or tissue regeneration.

Keywords: *Dental Pulp Stem Cell; Bone Morphogenic Protein; Vascular Endothelial Growth Factor; Biomedical Engineering*

Abbreviations

DPSC: Dental Pulp Stem Cell; BMP: Bone Morphogenic Protein; VEGF: Vascular Endothelial Growth Factor; IRB: Institutional Review Board; OPRS: Office for the Protection of Research Subjects; UNLV: University of Nevada, Las Vegas; SDM: School of Dental Medicine;

DMSO: Dimethyl Sulfoxide; FBS: Fetal Bovine Serum; RMPI: Roswell Park Memorial Institute; ISCT: International Society for Cellular Therapy; rDT: Rapid Doubling Time; iDT: Intermediate Doubling Time; sDT: Slow Doubling Time

Introduction

Stem cell research has recently described many new sources and clinical applications that are now available with many new potentially exciting discoveries [1,2]. For example, many researchers have demonstrated therapeutic and clinical applications for autologous dental pulp stem cells (DPSC) for a number of disease states [3-5]. These efforts have placed renewed focus on the mechanisms responsible for directional and specific differentiation for DPSC explants and isolates [6-8].

Many methods have been evaluated for lineage specific differentiation cues specific to DPSC, including three-dimensional bioscaffolds and modulated laser irradiation [9,10]. However, due to the specialized nature of these approaches, more practical and widespread technologies have been deployed to facilitate these applications [11-13]. New research has revealed that more pragmatic approaches to DPSC expansion and bioengineering may have the potential to revolutionize these methods and approaches using more commonly available biomolecules and growth factors [14-16].

Many new studies now describe the potential to use DPSC for bone tissue regeneration and bioengineering [17-19]. These studies describe the use of bone morphogenic protein (BMP) as a powerful growth factor that can modulate and regulate MSC (and potentially DPSC) differentiation [20-22]. In fact, osteoblastic, odontoblastic, and osteogenic differentiation of DPSC using BMP has now become a more focused research strategy [23-25].

Recent efforts from this group have revealed that specific subpopulations of DPSC may be responsive to BMP-induced phenotypic changes [26]. Moreover, other growth factor stimuli have also been demonstrated to induce similar changes to DPSC *in vitro* [27]. Based upon these observations, the primary objective of the current study was to evaluate the potential for BMP (alone and in combination) to induce changes to DPSC phenotypes.

Materials and Methods

Study approval

This study was retrospective in nature, utilizing previously isolated and characterized DPSC explants and isolates [28-30]. The original protocol for the collection and storage of DPSC was reviewed and approved by the Institutional Review Board (IRB) and the Office for the Protection of Research Subjects (OPRS) under OPRS#0907-3148 "Isolation of Non-Embryonic Stem Cells from Dental Pulp". The current retrospective analysis was reviewed and approved under OPRS#763012-1 "Retrospective analytsis of dental pulp stem cells (DPSC) from the University of Nevada, Las Vegas (UNLV) School of Dental Medicine (SDM) pediatric and clinical population".

DPSC culture

DPSC explants and isolates were cryopreserved in 10% dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) at -80°C. Each DPSC isolate was originally given an non-duplicated numerical identifier to prevent research bias and to remove any patient identifying information associated with the original sample collection protocol. No patient information was available to any research team member. Cells were thawed and cultured using Roswell Park Memorial Institute (RMPI) medium, supplemented with 10% FBS and 1% antibiotic solution consisting of Penicillin-Streptomycin in a humidified tissue culture incubator at 37°C with 5% CO_2.

In brief, DPSC cells were screened according to the International Society for Cellular Therapy (ISCT) criteria and were found to express CD90 or CD105 and did not express CD45 or CD34 [31]. Each DPSC was cultured with the frequency of passage or doubling time noted, as previously described [28-30]. In brief, DPSC were characterized as exhibiting a rapid doubling time (rDT) of approximately 1 - 2 days, an intermediate doubling time (iDT) of 4 - 6 days, or a slow doubling time (sDT) of approximately 10 - 12 days. The previous work from this group established that sDT DPSC isolates were responsive to BMP, whereas rDT and iDT DPSC isolates were not [26]. Based upon this information, sDT DPSC isolates were selected for inclusion in the current study with iDT DPSC isolates as negative controls.

Cell viability

Cells were plated in 96-well assay plates with and without the addition of BMP-2 at a concentration of 10 ng/mL for three days. Parallel experiments were also plated using VEGF at this same concentration, with additional wells containing both BMP-2 and VEGF in combination. Cell viability was assessed using the Trypan Blue exclusion assay and a BioRad TC20 cell counter. Total and live cell number (as well as percentage of viable cells) were noted for each experimental condition and control. Each experiment was done in triplicate (n = 3 rows of n = 8 wells, total per experimental condition n = 24).

Cell proliferation

Following viabilty testing, cells were fixed in 10% formalin and stained using Gentian Violet. Each plate was examined using a BioTek 808x 96-well plate reader at 630 nm to evaluate the total confluence and cell number in each experimental condition (control, growth factor). Results were plotted and graphed and differences between control and experimental conditions were evaluated using two-tailed t-tests, with an alpha level, a = 0.05 to determine statistical significance.

Results

The results of this analysis revealed that BMP-2 and VEGF have specific effects on sDT DPSC viability (Figure 1). More specifically, the baseline viability for the sDT DPSC isolates (average 27.1%, range 24 - 31%) was significantly increased with the administration of BMP-2 (average 49%, range 46.7% - 51.3%), p = 0.00147. In addition, the combination of BMP-2 and VEGF was sufficient to increase viability to an even higher level (64%, range 57.7% - 70%), which was significantly higher than the baseline controls and the administration of only BMP-2, p = 0.00412. However, the administration of VEGF in the absence of BMP-2 did not significantly affect cellular viability among any of the sDT DPSC isolates (average 27.7%, range 24.6% - 31.3%) compared with the negative control, p = 0.688.

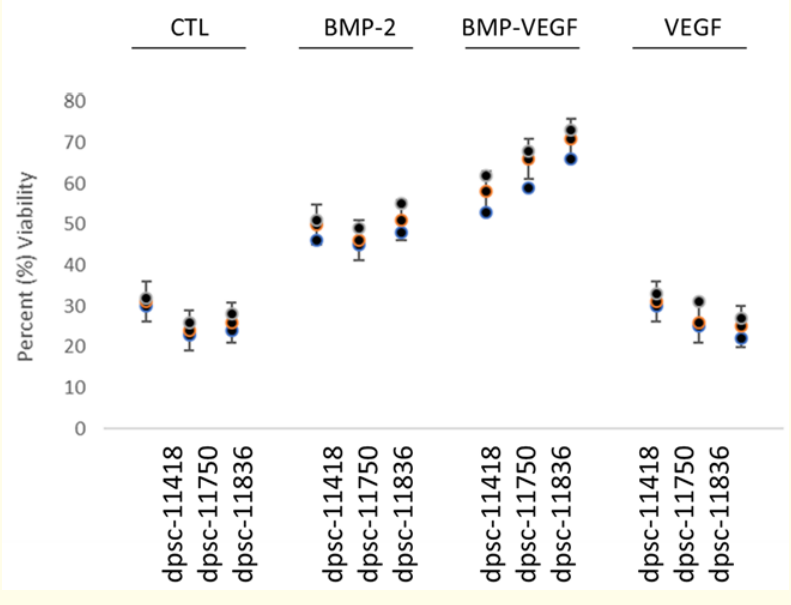

Figure 1: *Viability of sDT DPSC isolates following growth factor administration. Average viability of sDT DSPC isolates 27.1% (dpsc-11418, dpsc-11750, dpsc-11836) was significantly higher under BMP-2 (49%), p = 0.00147; with even higher viability with BMP-2 and VEGF in combination (64%), p = 0.00412. However, VEGF in the absence of BMP-2 did not affect viability (27.7%) compared with negative controls, p = 0.688.*

The analysis of these data with the iDT DPSC isolates revealed that neither BMP-2 or VEGF have specific effects on iDT DPSC viability (Figure 2). More specifically, the baseline viability for the iDT DPSC isolates (average 33.4%) was comparable with the administration of BMP-2 (average 32.4%), p = 0.4313. In addition, the combination of BMP-2 and VEGF also had no significant effect on viability (34.3%), p = 0.5557. Finally, the administration of VEGF in the absence of BMP-2 also did not significantly affect cellular viability among any of the iDT DPSC isolates (average 34.8%) compared with the negative control, p = 0.2621.

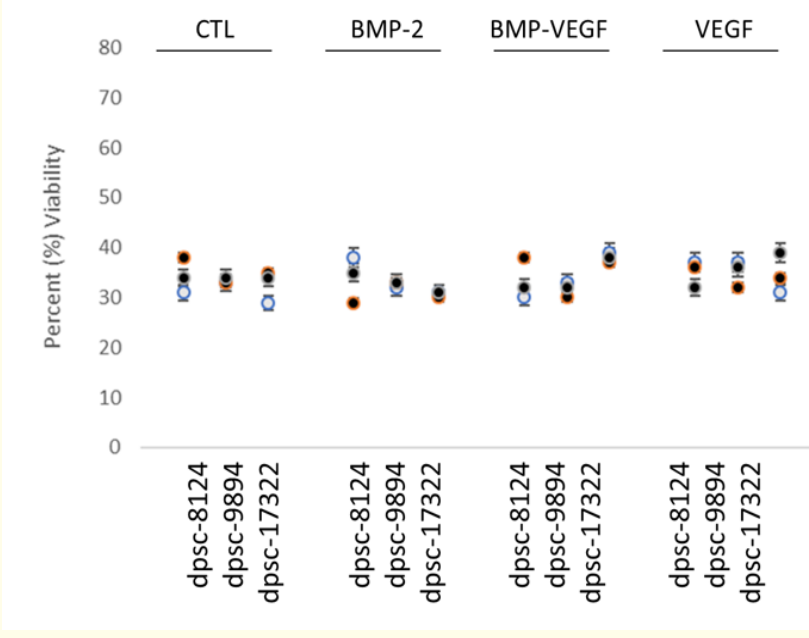

Figure 2: *Viability of iDT DPSC isolates following growth factor administration. Average viability of iDT DPSC isolates (33.4%) was comparable with BMP-2 administration (32.4%), p = 0.4313, VEGF administration (34.8%), p = 0.2621 and the combination of BMP-2 and VEGF in combination (34.3%), p = 0.5557.*

To determine if the effects of BMP-2 and VEGF also induced changes to cellular proliferation, 96-well three-day proliferation assays were also performed (Figure 3). These data demonstrated that administration of BMP-2 was sufficient to increase growth among the sDT DPSC isolates by nearly two-fold (baseline average absorbance 0.325, BMP-2 0.651), which was statistically significant, p = 0.00065. Furthermore, variable but significantly increased growth was also observed with the combined administration of BMP-2 and VEGF (average 0.895), p = 0.00091. However, VEGF administration was not sufficient to induce any significant or measurable changes to sDT growth among the sDT DPSC isolates (average absorbance 0.334), p = 0.759.

To more closely evaluate the variable changes induced by concomitant BMP-2 and VEGF administration, these data were re-graphed to analyze these results sorted by sDT DPSC isolate (Figure 4). These data revealed a marked, variable response among the sDT DPSC isolates. For example, the administration of BMP-2 increased growth among dpsc-11418 by 91% while the combined administration of BMP-2 and VEGF increased growth by 139% - clearly suggesting an additive or synergistic effect.

However, the administration of BMP-2 increased growth among dpsc-11750 by nearly 131%, while the combined administration of BMP-2 and VEGF increased growth by only 71.3%. This may suggest VEGF exerts a contradictory or negative effect on the growth-enhancing effects of BMP-2 with this DPSC isolate.

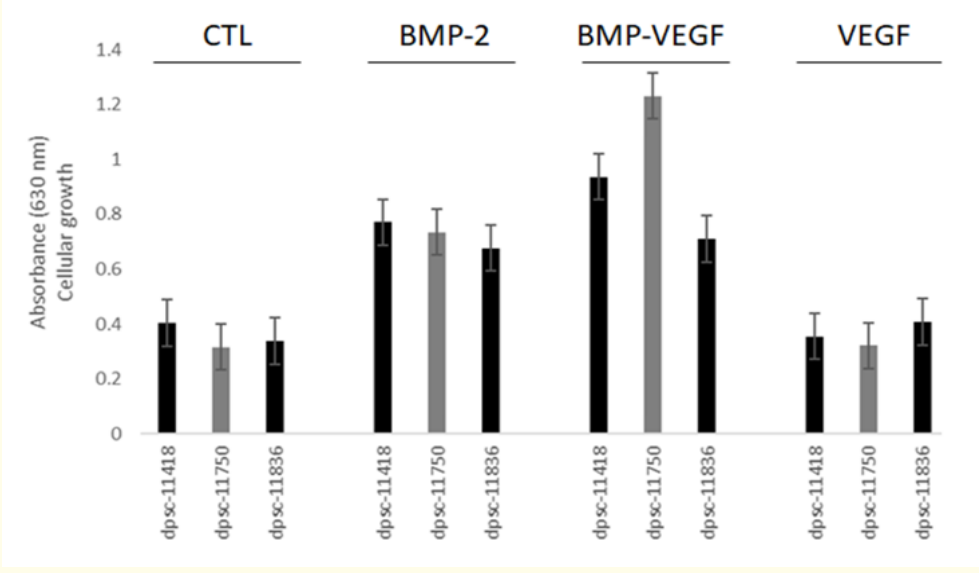

Figure 3: *Changes to sDT DPSC growth following growth factor administration. Addition of BMP-2 was sufficient to induce significant increases in growth among the sDT DPSC (average: 0.651) compared with baseline controls (average: 0.325), p = 0.00065. Variable increased growth was observed with BMP-2 and VEGF (average 0.895), p = 0.00091 with no changes under VEGF administration (average 0.334), p = 0.759.*

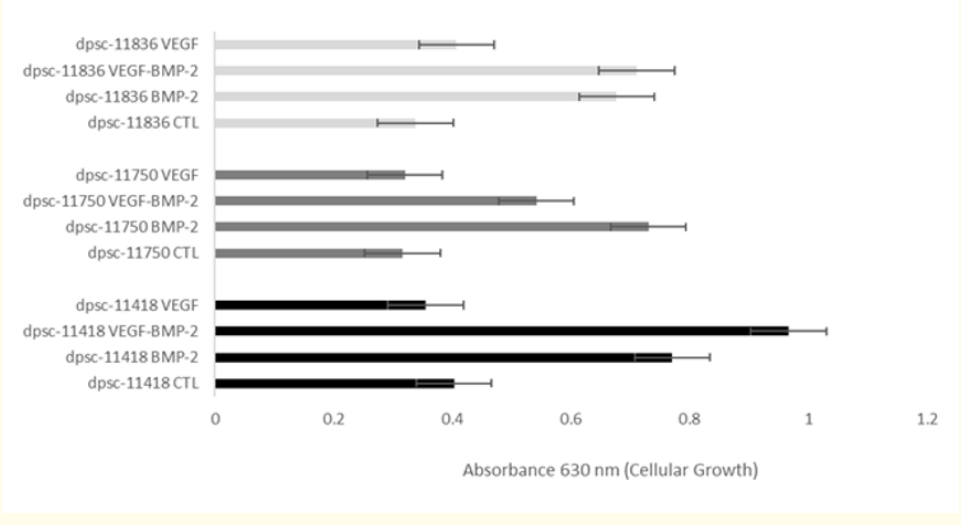

Figure 4: *Variable growth induced by concomitant BMP-2 and VEGF administration sorted by sDT DPSC isolate. Among dpsc-11418 cells, BMP-2 increased growth by 91%, while BMP-2 and VEGF increased growth by 139%. Among dpsc-11750, BMP-2 increased growth by 131%, while BMP-2 and VEGF increased growth by only 71.3%. Finally, BMP increased growth by 99% among dpsc-11836, which was similar to combined administration of BMP-2 and VEGF (105%). No significant changes from controls were observed with the administration of VEGF.*

Finally, the administration of BMP increased growth by nearly double, or 99%, among dpsc-11836, which was similar to the growth observed under combined administration of BMP-2 and VEGF (105%), suggesting that VEGF may not be exerting any additional effects on growth in this DPSC isolate.

These growth assays were also performed to evaluate whether growth factor administration affected iDT DPSC growth following growth factor administration (Figure 5). These data demonstrated that baseline growth or absorbance from negative controls (average 0.301) was not significantly different among the BMP-2 experimental group (average 0.303, p = 0.911). Moreover, the combined administration of both BMP-2 and VEGF also did not exhibit any significant effects on iDT DPSC growth (average 0.287, p = 0.364). Finally, the administration of VEGF alone did not induce any significant changes from baseline controls (average 0.312, p = 0.289).

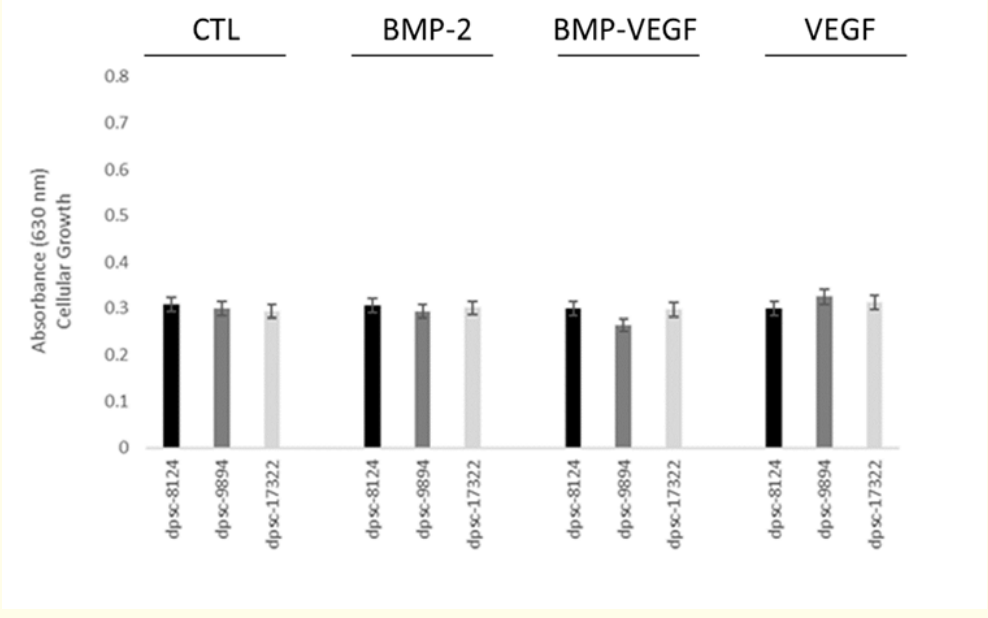

Figure 5: *Analysis of iDT DPSC growth following growth factor administration. Baseline growth or absorbance from negative controls (average 0.301) was not significantly different among the BMP-2 (average 0.303, p = 0.911), combined BMP-2 and VEGF (average 0.287, p = 0.364), or VEGF (average 0.312, p = 0.289) experimental groups.*

To more closely evaluate these effects on sDT DPSC isolates photo microscopy was performed (Figure 6). These data demonstrated both the increased cell number under BMP-2 (C, G, K) and combined VEGF-BMP-2 administration (D, H, L). No obvious changes to cellular number or morphology was apparent among the sDT DPSC isolates under VEGF administration alone (B, F, J) compared with non-treated controls (A, E, I).

Discussion

The goal of the current study was to evaluate the potential for BMP (alone and in combination) to induce changes to specific DPSC phenotypes. The results of this study clearly demonstrated that one subset of DPSC isolates (sDT) exhibited significant increased viability under both BMP-2 and combined VEGF-BMP-2 administration. Differential and variable increases in cell growth were observed among the corresponding slow DPSC isolates, which suggests more complex relationships between growth and growth factor administration may be present.

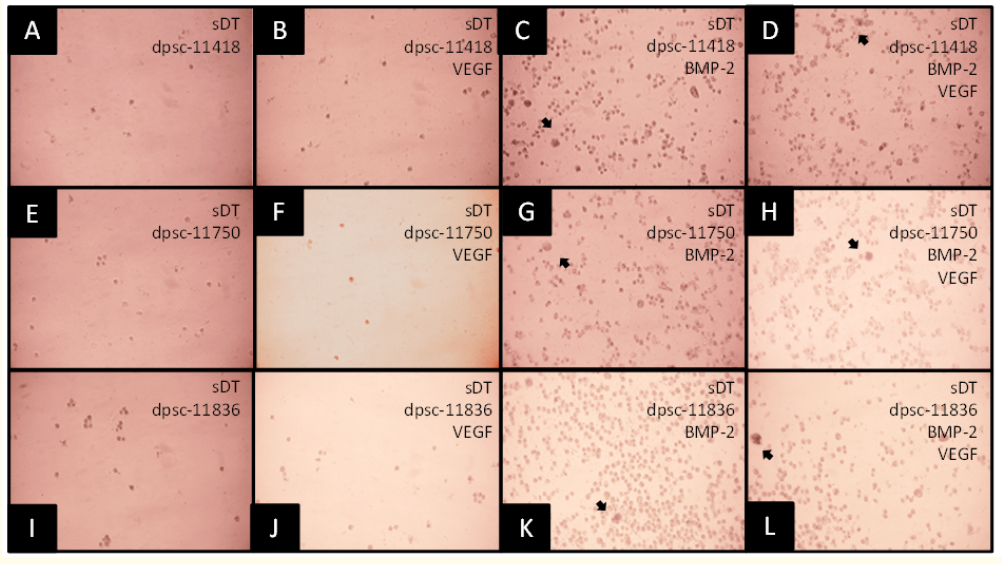

Figure 6: *Microscopy of sDT DPSC under growth factor administration. No apparent differences were observed between non-treated (control) cells (A, E, I) and VEGF (B, F, J). However, increased cell number and morphologic changes were observed among sDT DPSC isolates under BMP-2 (C, G, K) and combined VEGF-BMP-2 administration (D, H, L).*

However, no similar effects were observed with the other subset of DPSC isolates (iDT) and no effects on either growth or viability were observed under VEGF administration alone, which suggests these effects may be specific to the sDT DPSC isolates and may restricted to either BMP-2 or BMP-2 in combination with VEGF.

These data support other observations of diverse and differential effects of BMP-2 on other mesenchymal stem cells or MSCs [32,33]. In addition, the only other study to evaluate BMP-2 and VEGF administration in DPSC also found differential and diverse effects of these growth factors, both alone and in combination - which may suggest more complicated and complex relationships between the underlying DPSC biology and growth factor-induced phenotypes [34].

Although these data provide novel observations that may indicate future directions for research endeavors, there are some limitations that should also be discussed. First and most importantly, only a small number of DPSC isolates were available to this research team. A more thorough and comprehensive examination of other DPSC isolates may reveal other pertinent information regarding pluripotency and DPSC phenotypes, which could not be explored within the limited scope of this project [35,36].

Conclusion

Although only a limited number of DSPC isolates could be evaluated, the data from this study revealed a strong and significant change to sDT DPSC phenotypes (growth and viability) under BMP-2 administration (either alone or in combination with VEGF). These changes appear to be restricted to this subpopulation of DPSC, which may provide some insight into the mechanisms and underlying biology responsible for these observations. Future studies may be needed to evaluate the potential for BMP-2 and other growth factors to induce DPSC differentiation and lineage-specific phenotypic changes for bioengineering applications or tissue regeneration.

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Authors' Contributions

JC and EM performed the experimental protocols and data generation. KK was responsible for project design and supervision. All authors contributed to this manuscript and have read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.

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