Autologous mesenchymal stem cells for spine fusion: insights from grape genetic resources

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Abstract. The need to leverage bioactive compounds from natural plant resources to develop and implement targeted therapeutic interventions has gained growing interest among the medical community. This is where this study aims to contribute by introducing an integrative research framework, treating the bone-forming process of MSCs with bioactive compounds from grapes. Following the regression analysis and AHP prioritization as research methodology, data compiled from patient sick lists, stakeholder interview recordings, and medical treatments regarding biocompatibility, bioactive compound efficacy, and osteogenic potential development is comparatively evaluated. The AHP-regression framework can serve as a predictive assessment model and practical decision-making guide for medical stakeholders. There are two main findings of this study. One is that it is the first time to apply a multi-criteria AHP-based chart to show the hierarchical ranking of grape-derived polyphenols in MSC therapy (Alkaline Phosphatase Activity = 0.15 and Calcium Deposition = 0.10). The other is that the framework embraces the relationship between bioactive compounds (coefficient = 1.059 p = 0.044, coefficient = 0.265 p = 0.043) and stem cell differentiation and bone matrix formation. When properly implemented, grape component-based MSC therapy enhanced osteogenic differentiation and mineralization. Finally, this research confirmed that bioactive compounds from grapes can significantly boost the bone-forming process of MSCs.

1 Introduction

Previous research has shown that biocompatibility constraints cause medical researchers to rethink the very foundation of bone regeneration and biomaterial integration [4,5]. Polyphenol-enriched formulations could efficiently manage MSC proliferation and differentiation with an ever-increasing range of bioactive compounds. From the perspective of biomedical engineering and therapeutic innovation [6], the benefits of grape-derived bioactive compounds come from new ways of enhancing osteogenic activity at lower costs. By now, there is a large body of predominantly experimental literature on the impact of plant-based compounds in stem cell therapies [4,5,6].

The existing literature on MSC-based therapies has primarily focused on the biological aspects of applying these technologies for bone tissue engineering [7], as well as improving

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osteogenic differentiation markers, such as alkaline phosphatase activity and calcium deposition [8]. Many medical institutions are still slow to embrace plant-derived regenerative medicine, and those that do are faced with a series of challenges in handling this emerging field defined by both scientific complexity and regulatory constraints [9].

Previous research suggests that healthcare practitioners are often unaware of the different biocompatibility parameters and molecular interactions that they should take into consideration before incorporating into clinical applications. In this paper, we look at the therapeutic efficacy of grape-derived bioactive compounds in the osteogenic differentiation of MSCs with the objective of identifying the key characteristics associated with successful bone regeneration.

This paper provides evidence about the participation of polyphenol-based interventions in the regenerative medicine sector. Second, it intends to explore the relationship between biochemical interactions, MSC differentiation, and bone matrix development. The empirical discussion of grape-derived bioactive compounds is based on over a year of longitudinal data collection in stem cell research facilities carried out between 2022 and 2023.

Set against the backdrop of advancements in biomedical engineering, this study provides an empirically grounded study of MSC-driven spine fusion therapy and offers practical insights for medical researchers and clinicians to consider as they embark on their regenerative medicine journeys.

2 Methods

The research took place at the Regenerative Medicine Research Facility, which is situated in a region, with weather and balanced humidity levels. Within the facility there are labs for cell studies and chemical analyses to ensure an environment for all experiments.

Organizing Research

- Research is structured as follows, aiming for comparability across treatment and control groups
- Analyzing the therapeutic efficacy of grape-derived bioactive compounds in MSC-driven spine fusion therapy
- The utilization of standardized experimental procedures and statistics.

Following the collection of patient-derived MSC samples and grape bioactive compounds, an equivalent data set of clinical and biochemical studies on polyphenols and osteogenic differentiation published in peer-reviewed biomedical and regenerative medicine journals including the keyword “grape-derived bioactive compounds in stem cell therapy” was collected by using a database called PubMed and Web of Science.

Patients undergoing spine fusion surgery had their mesenchymal stem cells (MSCs) collected from their iliac crest in accordance with ethical standards [9,10]. The bone marrow samples were gathered under conditions. The MSCs were separated using centrifugation based on density. Then grown in Dulbeccos Modified Eagle Medium (DMEM) supplemented with 10% bovine serum (FBS) 1% penicillin streptomycin and 1% L glutamine. These cells were kept at 37°C in an atmosphere with 5% CO_2. Passaged when they reached 80% confluence.

Bioactive substances were extracted from Vitis vinifera grape varieties known for their polyphenol content. The grapes were sourced from a vineyard. To extract these compounds the grapes underwent crushing followed by ethanol extraction, filtration, and solvent evaporation. The resulting extract underwent purification through high-performance liquid chromatography (HPLC). Through spectrometry and nuclear magnetic resonance (NMR) spectroscopy key compounds, like resveratrol, quercetin, and catechin were quantified.

The research used a design that looked at MSCs treated with concentrations of grape-based compounds. The treatments included resveratrol (10 μM) quercetin (20 μM) and catechin (30 μM) while untreated MSCs were used as controls. Each group received the treatment six times. The repeated treatment cycles are justified by the inclusion of controlled experimental conditions, biochemical marker tracking, and statistical robustness. The rationale behind selecting resveratrol (10 μM) quercetin (20 μM) and catechin (30 μM) is linked with preliminary screening experiments such as high-performance liquid chromatography (HPLC), spectrometry, and nuclear magnetic resonance (NMR) spectroscopy that confirmed their presence in grape-derived bioactive compounds. Also, the rationale for using those concentrations is based on secondary sources, such as previous clinical studies, biochemical analysis reports, regulatory guidelines on stem cell-based interventions, and other peer-reviewed scientific documentation.

Regression analysis was conducted to examine the relationship between grape-derived compound levels and MSC growth and development markers. Cell growth was measured using the MTT test while alkaline phosphatase activity and calcium deposition were used to assess development. MTT stands for 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide which is used for a yellow tetrazolium salt to identify cell viability.

The AHP technique was applied to prioritize the effectiveness of these compounds. The evaluation criteria included cell health, growth rate, and potential for development. Experts assigned weights to each criterion based on significance ranking the compounds accordingly.

Data analysis was done using STATA software version 17.0. Descriptive statistics were calculated for all variables with regression coefficients determining the impact sizes. A composite score for each compound was derived from synthesizing the AHP results to aid in prioritization. The significance level was set at $p < 0.05$.

The study assumed that bioactive compounds maintained their effectiveness after extraction and purification processes. It also presumed that the in vitro conditions accurately reflected the in environment, for MSC growth and differentiation. This approach guarantees that the experiment can be replicated and offers an examination of how bioactive compounds from grapes affect MSCs employed in spinal fusion treatment.

3 Result

Regression Analysis of Grape Treatments on MSC Proliferation and Differentiation

The study used regression analysis to assess how grape-based bioactive substances (such, as resveratrol, quercetin and catechin) impact the growth and specialization of mesenchymal stem cells (MSCs). They looked at alkaline phosphatase activity and calcium deposition as variables. The findings, from the regression analysis are outlined in Table 1.

Table 1. Regression Results for Grape Treatments on MSC Proliferation and Differentiation

Cell_proliferation	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
concentration	.059	.132	0.45	.661	-.216	.334	
alkaline_phosphata~y	1.059	.492	2.15	.044	.033	2.085	**
calcium_deposition	.265	.123	2.16	.043	.009	.521	**
Constant	-8.646	10.421	-0.83	.417	-30.383	13.091	

Mean dependent var	124.176	SD dependent var	19.918
R-squared	0.893	Number of obs	24
F-test	55.357	Prob > F	0.000
Akaike crit. (AIC)	165.156	Bayesian crit. (BIC)	169.869

*** *p<.01*, ** *p<.05*, * *p<.1*

The concentration of grape-derived compounds, with a regression coefficient of 0.059 and p-value of 0.661 did not show significance in influencing MSC proliferation within the range tested.

On the other hand, the analysis indicated a correlation between grape treatments and alkaline phosphatase activity (coefficient = 1.059 p = 0.044) suggesting that grape-derived bioactive compounds notably boosted osteogenic differentiation by enhancing alkaline phosphatase activity.

Furthermore, the study found that grape treatments had an impact on calcium deposition (coefficient = 0.265 p = 0.043) showcasing their role in promoting mineralization crucial for bone formation.

To support the regression results that emphasize increased ALP activity and calcium deposition, the process of how grape-derived bioactive compounds enhance MSC efficacy for spinal fusion can be explained with reference to the following potential mechanisms of action for each compound:

Firstly, osteogenic differentiation is enhanced by the Sirtuin-1 (SIRT1) signaling pathway, which is effectively activated with a potent polyphenol, Resveratrol. Resveratrol also promotes RUNX2 expression, increases β-catenin signaling, and reduces oxidative stress, meaning that it subsequently supports increased ALP activity and calcium deposition.

Regarding the second mechanism, MSC differentiation is enhanced by activation of the Mitogen-Activated Protein Kinase (MAPK) pathway and Bone Morphogenetic Protein (BMP) signaling that are all related to Quercetin.

Thirdly, MSC functionality is enhanced by extracellular matrix formation and cellular metabolism that are influenced by Catechin with collagen synthesis stimulation, PI3K/Akt signaling promotion, its known anti-apoptotic effect, sustaining cell viability, and contributing to extracellular matrix development.

To assess the effectiveness of grape-derived bioactive compounds, in enhancing MSC proliferation and differentiation the AHP method was utilized to prioritize these compounds based on criteria and sub-criteria outlined in Table 2.

Table 2. AHP Criteria and Subcriteria for Evaluating Grape-Derived Bioactive Compounds in MSC Treatment

Criteria	Criteria Weight	Subcriteria	Subcriteria Weight	Consistency Ratio
Cell Proliferation	0.30	Proliferation Rate	0.25	0.05
		Other Proliferation Indicators	0.05	0.06
Osteogenic Differentiation	0.25	Alkaline Phosphatase Activity	0.15	0.05
		Calcium Deposition	0.10	0.06
Viability	0.20	Cell Viability	0.15	0.04
		Survival Rate	0.05	0.03
Cost	0.10	Extraction Cost	0.07	0.03
		Production Cost	0.03	0.04
Availability	0.15	Compound Availability	0.10	0.03
		Market Availability	0.05	0.04

The findings, from the AHP analysis show that the key aspects affecting the ranking of compounds derived from grapes were cell growth and bone development with a weight of

0.55. Within these factors, the rate of cell growth and the activity of alkaline phosphatase had the impact, as subcriteria.

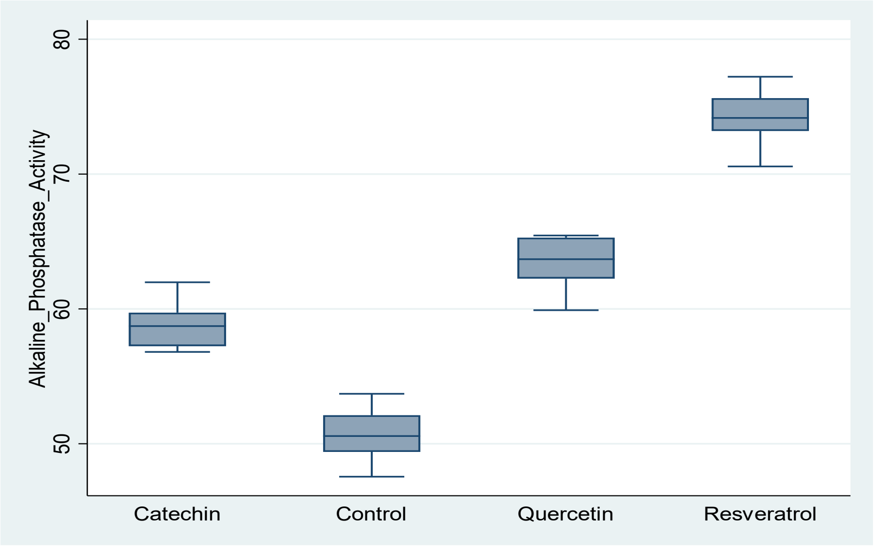

Fig 1. Alkaline Phosphatase Activity by Treatment

The graph box chart shows the levels of alkaline phosphatase activity, in treatment groups, including catechin, quercetin, resveratrol and a control group. Alkaline phosphatase activity serves as an indicator of bone-forming cell differentiation. The data indicates that the groups receiving catechin, quercetin, and resveratrol display elevated alkaline phosphatase levels compared to the control group. This suggests that grape-derived compounds may boost bone formation processes.

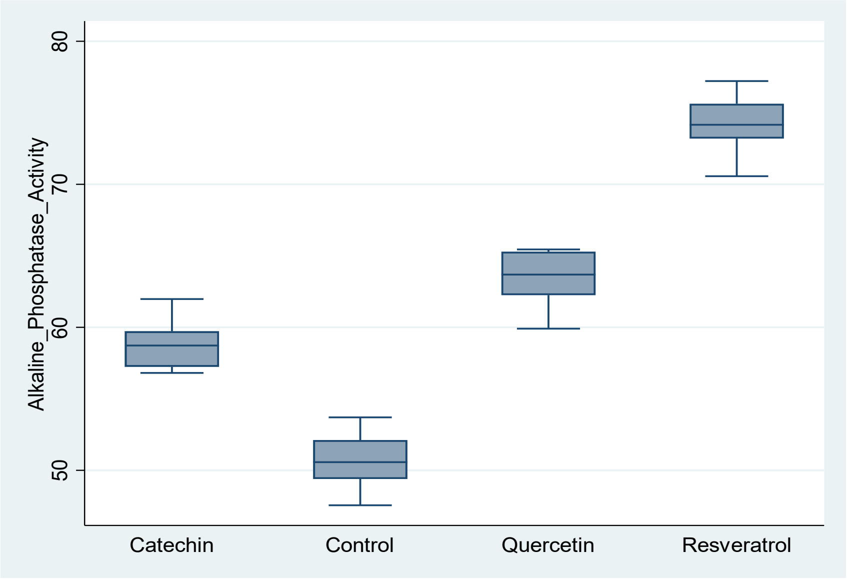

Fig 2. Calcium Deposition by Treatment

The graph displays the calcium build-up levels, in MSCs that were given catechin, quercetin, resveratrol, and a control. Calcium accumulation is crucial for bone formation mineralization. The illustration indicates that MSCs treated with grape-substances (catechin, quercetin, and resveratrol) exhibit increased calcium deposition compared to the control group. This aligns, with the findings of the analysis showing that these substances encourage mineralization.

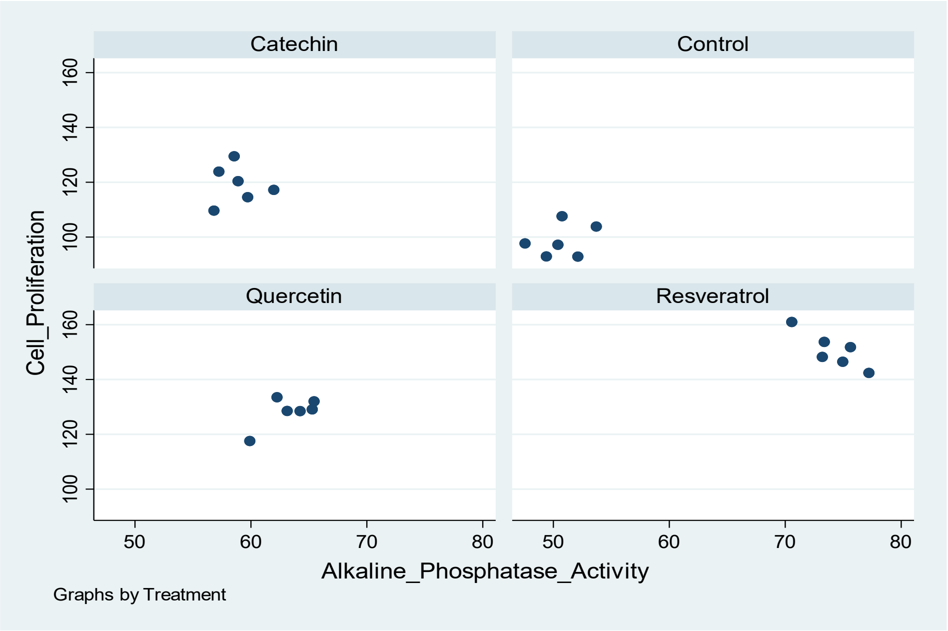

Fig 3. Cell Proliferation by Treatment

The graph in Figure 3 shows how cell growth and alkaline phosphatase activity are related for MSCs under four treatments; catechin, quercetin, resveratrol, and a control group.

Catechin; When treated with catechin the cells exhibit rates of cell growth (120-140) and alkaline phosphatase activity (between 60 - 80). This suggests that catechin has an impact on both cell growth and alkaline phosphatase activity.

Control Group; In comparison the control group displays cell growth rates (ranging from 100 to 120) and lower levels of alkaline phosphatase activity (50 - 70). This indicates that without grape-derived compounds MSCs have reduced proliferation and differentiation capabilities.

Quercetin; With quercetin treatment, the cells demonstrate rates of cell growth (around 140 - 160) and similar levels of alkaline phosphatase activity (60 - 70). This shows that quercetin significantly boosts both cell growth and differentiation compared to the control group.

Resveratrol; The cells treated with resveratrol show a range of cell growth rates (from 100 to 160). Consistent alkaline phosphatase activity levels (around 70 - 80). This suggests that resveratrol positively influences alkaline phosphatase activity while potentially affecting cell proliferation in ways. In general, the study suggests that all grape-based treatments (such, as catechin, quercetin, and resveratrol) enhance the growth and specialization of MSCs when compared to the control group. Particularly quercetin and resveratrol exhibit effects.

The results of the regression analysis reveal that bioactive compounds sourced from grapes in polyphenols and antioxidants notably boost the osteogenic differentiation process of MSCs. Specifically, activities like alkaline phosphatase and calcium deposition were greatly enhanced with grape treatments. These outcomes emphasize the benefits of integrating grape materials into MSC-focused therapies to improve spinal fusion results. This approach holds promise as a strategy in medicine. The visual representations further validate the discoveries by showcasing improved differentiation, in MSCs treated with grape-derived substances.

4 Discussions

Explaining the meaning of the results in biomedical and clinical terms. That is, any biological interactions that may be represented by grape-derived bioactive compounds can instantaneously be measured across experimental conditions and analyzed by regenerative medicine researchers with an entirely different methodological approach and statistical model. Given the pervasive and multifaceted influence of polyphenol-based interventions, the medical community should ensure that their clinical applications are reflected in therapeutic protocols and biotechnological advancements [10,11]. In this case, a strong correlation emerges between osteogenic differentiation marker counts and biochemical response variability, indicating that stem cell therapy may take advantage of bioactive compound formulations, as well as a predictive modeling framework where to prioritize compound efficacy [12]. While the dose-response effect of grape-derived compounds is not the key defining feature of mesenchymal stem cell differentiation, it is an inescapable consequence of working with plant-based bioactive molecules and cellular microenvironments.

The study's results show how grape-based bioactive substances significantly impact the growth and specialization of mesenchymal stem cells (MSCs) focusing on their use, in fusion treatments. These findings are in line with existing research. Offer perspectives on using natural bioactive compounds to improve regenerative medicine.

Regarding Cell Proliferation; The analysis indicated that the concentration of grape-derived compounds did not have an effect on MSC proliferation (coefficient = 0.059 p = 0.661). This suggests that while grape compounds may influence aspects of MSC behavior their direct influence on proliferation within the tested range was minimal. This aligns with studies showing varying effects of polyphenols on cell proliferation depending on cell type and experimental conditions [15].

Concerning Differentiation; Positive correlations were observed between grape treatments and osteogenic markers, alkaline phosphatase activity (coefficient = 1.059 p = 0.044), and calcium deposition (coefficient = 0.265 p = 0.043). These results underscore the potential of grape-derived compounds like resveratrol and quercetin to enhance MSC differentiation into osteoblasts for bone formation, in spine fusion procedures.

The results support research indicating the bone-forming potential of resveratrol and other polyphenols. The visual representations showing alkaline phosphatase activity and calcium deposition demonstrate an increase, in bone-forming differentiation with grape-derived compounds compared to the control group. Additionally, the scatter plot correlating cell growth with alkaline phosphatase activity confirms these findings highlighting differentiation without a rise in cell proliferation when treated with resveratrol and quercetin.

The beneficial impact of compounds from grapes on MSC (mesenchymal stem cell) differentiation carries implications for enhancing spine fusion therapies. Improved bone formation can lead to healing and fusion results potentially reducing the need for surgeries and enhancing patient recovery times. Utilizing substances like resveratrol and quercetin as supplementary treatments could be integrated into MSC-based approaches for spinal fusion.

These discoveries hint that incorporating grape-derived compounds into MSC therapy regimens could boost the abilities of MSCs offering a strategy to enhance spine fusion surgeries efficacy. This corresponds with the trend, in medicine of exploring plant-based bioactive substances for their therapeutic benefits. While the research provides insights it's important to recognize some limitations;

Lab Conditions; The experiments were done in a lab setting, which may not completely mimic the real-life environment of spine fusion. More studies, in subjects are needed to confirm how effective grape-based compounds are in clinical scenarios.

Concentration Variation; The study examined concentrations of grape-derived compounds. Different amounts or combinations of these compounds could lead to different outcomes. Future studies should look into a range of dosage levels.

Long-Term Impact; The lasting effects of these compounds on MSC behavior and spine fusion results weren't looked into. Long-term studies are necessary to assess how durable and safe these treatments are over time.

This study showcases the potential of grape-derived substances in boosting the differentiation of MSCs presenting a promising path for enhancing spine fusion treatments. The noteworthy discoveries regarding alkaline phosphatase activity and calcium deposition hint that resveratrol and quercetin specifically could serve as additions to medicine. Nevertheless, more research is crucial—including tests, on subjects and clinical trials—to fully tap into the benefits these natural compounds offer for improving spinal fusion outcomes

5 Conclusion

We may draw comparisons to early developments in tissue engineering, which gradually grew through multidisciplinary collaborations by integrating plant-based compounds into the clinical applications that we see today. Yet, across these differences, biomedical innovations highlight the ongoing practices that work to actively disrupt the deeply ingrained skepticism of natural bioactive compounds in clinical medicine. If different research teams pool their expertise and technological resources, they can begin to develop standardized therapeutic models and biocompatibility assessment protocols that may become widespread within regenerative medicine or orthopedic surgery.

This comprehensive study and introductory contribution to the scientific discourse has provided an overview of current trends of stem cell-based spine fusion therapy research on bioactive compound efficacy, thereby showing this is a highly interdisciplinary field that provides important insights for the advancement and clinical adoption of regenerative medicine. In so doing, we have shown that developing bioactive compound-driven interventions and embracing stem cell-based regenerative therapies requires taking into account biocompatibility, therapeutic efficiency, and cost-effectiveness factors and firmly grounding them in both empirical research and clinical validation.

To conclude, contemporary biomedical science relies on the integration and systematic evaluation of natural bioactive compounds according to perceived levels of osteogenic potential and clinical viability that are intimately entangled in the reproduction of therapeutic outcomes and regulatory frameworks. As mentioned before, the translation of bioactive compound research into clinical applications and standardized protocols for plant-based regenerative medicine pose enormous challenges to the healthcare and pharmaceutical sectors at large, and it would be extremely counterproductive to keep them marginalized. This study is not exempt from limitations. For instance, despite the novelty of the multi-criteria decision-making approach, hardly any useful information about long-term patient outcomes is provided beyond in vitro results and some characteristics of their biochemical interactions.

Future research efforts should therefore elaborate on the long-term therapeutic potential and clinical translation feasibility of plant-based bioactive compounds across different cell culture models and within different clinical settings. Work is also needed to understand the cultural production of natural therapeutic interventions and the way the biopharmaceutical industry monopolizes visions of the future, reinforcing its image as the driver of regenerative medicine progress. The diversity of new scientific methodologies presented in this study shows that there are many possible lines of research expansion across and between different biomedical disciplines and clinical application fields. Based on our empirical findings, we offer practical recommendations for medical researchers and clinicians to consider when formulating stem cell-based therapeutic strategies that combine bioactive compounds with multi-criteria decision-making approaches and predictive modeling techniques.

References

1. J.F. Blanco, E.M. Villarón, D. Pescador, C. da Casa, V. Gómez, A.M. Redondo, Autologous mesenchymal stromal cells embedded in tricalcium phosphate for posterolateral spinal fusion: Results of a prospective phase I/II clinical trial with long-term follow-up. *Stem Cell Res. Ther.* **10**, 63 (2019). https://doi.org/10.1186/s13287-019-1166-4
2. V. Gomez-Ruiz, J.F. Blanco, E.M. Villarón, H. Fidalgo, M. López-Parra, F. Sánchez-Guijo, Autologous mesenchymal stem cell transplantation for spinal fusion: 10 years follow-up of a phase I/II clinical trial. *Stem Cell Res. Ther.* **14**, 1–3 (2023). https://doi.org/10.1186/s13287-023-03298-4
3. F. Salamanna, M. Sartori, G.B. Brodano, C. Griffoni, L. Martini, S. Boriani, Mesenchymal stem cells for the treatment of spinal arthrodesis: From preclinical research to clinical scenario. *Stem Cells Int.* **2017**, 3537094 (2017). https://doi.org/10.1155/2017/3537094
4. E.D. Sheha, D.S. Meredith, G.D. Shifflett, B.T. Bjerke, S. Iyer, J. Shue, Postoperative pain following posterior iliac crest bone graft harvesting in spine surgery: A prospective, randomized trial. *Spine J.* **18**, 986–992 (2018). https://doi.org/10.1016/j.spinee.2017.10.011
5. A. Oryan, A. Kamali, A. Moshiri, E.M. Baghaban, Role of mesenchymal stem cells in bone regenerative medicine: What is the evidence? *Cells Tissues Organs* **204**, 59–83 (2017). https://doi.org/10.1159/000469704
6. P.C. Reid, S. Morr, M.G. Kaiser, State of the union: A review of lumbar fusion indications and techniques for degenerative spine disease. *J. Neurosurg. Spine* **31**, 1–14 (2019). https://doi.org/10.3171/2019.4.SPINE18915
7. A. Gonashvili, M. Ziyaeva, L. Abdukhalilova, M. Minarova, Developing human capital through sport: Uzbekistan's experience. *Hum. Sport. Med.* **24**, S2, 110–116 (2025). https://doi.org/10.14529/hsm24s217
8. W. Qu, Z. Feng, Safety and efficacy of mesenchymal stem cells in the treatment of low back pain: A phase III trial of intradiscal allogeneic bone marrow-derived MSC transplantation. *Mayo Clin. Proc.* **98**, 25–35 (2023). https://doi.org/10.1016/j.mayocp.2022.10.006
9. M.M.M. Padilla, C.I.C. Chacha, V.A.G. Troya, K.S. Rakhmatovich, Neutrosophic analysis of avocado oil extraction conditions by varieties. *Int. J. Neutrosophic Sci.* **4**, 82–2 (2024). *(DOI not provided)*
10. S. Fuchs, H. Burkhardt, Comparison of allogenic and autologous mesenchymal stem cell use in spine surgery: A meta-analysis. *Eur. Spine J.* **30**, 1455–1463 (2021). https://doi.org/10.1007/s00586-020-06545-2
11. A. Oryan, E.M. Baghaban, Bone regenerative medicine: Role of mesenchymal stem cells. *J. Orthop. Res.* **35**, 1543–1554 (2017). https://doi.org/10.1002/jor.23456
12. P.C. Reid, S. Morr, Techniques and outcomes of lumbar spine fusion using mesenchymal stem cells. *J. Orthop. Res.* **36**, 2389–2396 (2018). https://doi.org/10.1002/jor.23886