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Comparative Efficacy of Autologous Platelet-Rich Plasma and Zinc Oxide Nanoparticles in Accelerating Cutaneous Wound Healing: A Randomized Controlled Study in Rabbits

Abdulaziz Faisal Alhussain[1], Saleh Abdmoeneem Alsayed[1], Mohamed Marzok[1*], Abdelrahman Hereba[2], Mohamed El-Sherif[3*] and Mohamed Abdelkawi[3]

[1] *Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, AlAhsaa 31982, Saudia Arabia.*

[2] *Department of Microbiology, College of Veterinary Medicine, King Faisal University, AlAhsaa 31982, Saudia Arabia.*

[3] *Department of Surgery, Faculty of Veterinary Medicine, New Valley University, Al Kharga, New Valley 27511, Egypt.*

Abstract

ADVANCED wound therapies are needed to enhance healing beyond standard care. We compared autologous platelet-rich plasma (PRP) with zinc oxide nanoparticles (ZnO NPs) for full-thickness cutaneous wound repair in rabbits. Thirty-six male New Zealand White rabbits (n = 12/group) received topical PRP, ZnO NPs, or saline on two standardized dorsal wounds (20 mm in diameter). Treatments were applied once daily for 5 days, then on alternate days until closure or Day 21. Both treatments accelerated healing versus control. On Day 15, mean closure was 95.6% (PRP), 89.7% (ZnO NPs), and 76.4% (control; all $P < 0.001$ vs control). Time to ≥99% closure was 17.3 ± 2.1 (PRP), 19.8 ± 2.4 (ZnO NPs), and 23.7 ± 3.2 days (control); PRP was faster than ZnO NPs ($P = 0.007$). PRP yielded higher angiogenesis and VEGF expression at Day 14 (198.5 ± 24.8 vs 168.9 ± 22.1; $P = 0.008$) with favorable modulation of TNF-α. Both modalities were safe; PRP showed greater overall efficacy.

Keywords: Platelet-Rich Plasma, Zinc Oxide Nanoparticles, Wound Healing, Angiogenesis, Growth Factors.

Introduction

Cutaneous wound healing involves coordinated cellular interactions across three overlapping phases: inflammation, proliferation, and remodeling [1]. Traditional wound management approaches often inadequately address the complex biochemical requirements for optimal tissue regeneration, particularly in challenging clinical scenarios [2]. This limitation has driven research into advanced therapeutic modalities that enhance natural healing through targeted cellular and molecular interventions [3, 4].

Platelet-rich plasma (PRP) has emerged as a promising autologous biological therapy that concentrates growth factors including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF) [5, 6]. Clinical evidence demonstrates PRP's ability to accelerate wound healing through enhanced angiogenesis, cellular proliferation, and collagen synthesis. Recent studies showed significant wound diameter reduction with PRP treatment, accompanied by increased VEGF and TGF-β expression and reduced inflammatory markers [7, 8].

Zinc oxide nanoparticles (ZnO NPs) represent a nanotechnology-based approach with distinct therapeutic advantages [9-11]. These nanoparticles exhibit multifaceted properties including antimicrobial activity, anti-inflammatory effects, and direct wound healing enhancement through improved cellular proliferation, migration, and angiogenesis

*Corresponding authors: Mohammed Marzok, E-mail: mmarzok@kfu.edu.sa, Tel.: 00966549184646
Mohamed El-Sherif, E-mail: mohamedelsherif@vet.nvu.edu.eg, Tel.: 01008467944

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[12-14]. Nanoparticle formulation provides enhanced bioavailability compared to bulk zinc compounds, potentially amplifying therapeutic effects while maintaining wound sterility [15].

Despite promising individual results for both therapies, direct comparative studies evaluating their relative efficacy remain limited. PRP leverages endogenous growth factor-mediated healing, while ZnO NPs act through direct cellular interactions and antimicrobial mechanisms [16]. Understanding their comparative effectiveness could inform clinical decision-making and identify potential synergistic opportunities.

Head-to-head evaluation is essential because PRP delivers a biologically rich autologous cocktail of growth factors, whereas ZnO NPs combine antimicrobial and pro-healing physicochemical effects; clinicians must often choose between biologic and nanotechnology-based options despite limited comparative evidence.

This research systematically compares the efficacy of topical autologous PRP versus zinc oxide nanoparticles on full-thickness cutaneous wounds in a standardized animal model, evaluating wound closure kinetics, histological healing quality, and molecular healing markers to provide evidence-based insights for wound care management.

Material and Methods

Study Design and Animals

This randomized controlled study compared autologous PRP and ZnO NPs efficacy using a standardized rabbit model. Thirty-six adult male New Zealand White rabbits (2.5-3.0 kg, 6-8 months) were randomly allocated into three groups (n=12): PRP treatment, ZnO NPs treatment, and saline control. Sample size was calculated to detect 20% difference in wound closure with 80% power (α=0.05).

Animals were housed individually under standard laboratory conditions (22±2°C, 12-hour light/dark cycle) with ad libitum access to standard pellet chow and water. All protocols were approved by the Institutional Animal Ethics Committee of King Faisal University, college of veterinary medicine.

Surgical Procedure

Two full-thickness excisional wounds (20 mm in diameter) were created using a sterile circular punch biopsy tool (Miltex, China), positioned symmetrically 5 cm from the midline and 3 cm apart.

Treatment Preparations

The final PRP preparation contained a minimum 3-fold increase in platelet concentration and was activated with a 1:10 ratio of 10% calcium chloride immediately before application.

ZnO NPs Synthesis: Zinc oxide nanoparticles were synthesized using sol-gel method with zinc acetate dihydrate (2.19 g) dissolved in deionized water (100 mL) at 60°C. Sodium hydroxide solution (0.8 g in 50 mL) was added dropwise under vigorous stirring, aged for 2 hours, then centrifuged and washed. The precipitate was dried at 80°C for 24 hours and calcined at 400°C for 2 hours. Final suspension was prepared at 1 mg/mL in sterile phosphate-buffered saline [18].

Treatment Protocol

Treatments were initiated immediately after wounding and applied once daily for the first 5 days, then on alternate days until complete closure or Day 21, whichever occurred first. All wounds were created using a 20-mm biopsy punch to standardize area, and a fixed volume of 0.2 mL per wound was applied at each treatment to ensure dose consistency.

Outcome Assessments

Morphometric Analysis: Wound healing was assessed through standardized digital photography at Days 0, 3, 6, 9, 12, 15, 18, and 21. Wound area was calculated using ImageJ software with percentage closure calculated as: [(Initial area - Current area) / Initial area] × 100.

Histological Analysis: Tissue samples (n=6 per group) were collected on Days 7, 14, and 21. Full-thickness samples including 2-mm surrounding tissue were fixed in 10% neutral buffered formalin, processed through standard protocols, and sectioned at 5μm thickness. Sections were stained with hematoxylin-eosin and Masson's trichrome. Histological parameters included epithelial regeneration, inflammatory infiltration, granulation tissue formation, angiogenesis, and collagen deposition using standardized scoring (0-12 scale).

Immunohistochemical Analysis: Expression of VEGF, TGF-β, TNF-α, and PDGF were evaluated using standard protocols with overnight primary antibody incubation at 4°C and DAB detection. Quantitative analysis employed semi-quantitative scoring (0-3 scale) with H-score calculation from five randomly selected high-power fields per section.

Statistical Analysis

Data analysis was performed using SPSS version 26.0 with continuous variables expressed as mean ± standard deviation. One-way ANOVA compared means between groups, with repeated measures ANOVA analyzing within-group changes over time. Post-hoc analysis used Tukey's HSD test. Statistical significance was set at $P < 0.05$.

Results

Macroscopic Wound Healing Assessment

Significant differences in wound healing progression were observed between treatment groups

starting from Day 6 post-wounding. Both PRP and ZnO NPs groups demonstrated accelerated wound closure compared to controls, with PRP showing the most rapid healing.

Complete wound closure (≥99% closure) was achieved significantly earlier in both treatment groups compared to controls. The PRP group achieved complete closure in 17.3 ± 2.1 days, the ZnO NPs group in 19.8 ± 2.4 days, and the control group in 23.7 ± 3.2 days ($P < 0.001$ for both comparisons with control). The difference between PRP and ZnO NPs groups was statistically significant ($P = 0.007$).

Relative reductions in time to ≥99% closure versus control were: PRP, (23.7 − 17.3) / 23.7 = 27.0%; ZnO NPs, (23.7 − 19.8) / 23.7 = 16.5% (reported as 16%).

Histological Analysis

Histological examinations revealed significant differences in tissue architecture and healing progression between groups.

Histological Wound Healing Scores

Collagen Assessment: Collagen deposition was significantly enhanced in both treatment groups. On Day 21, collagen density was 54.3 ± 6.8% (PRP), 48.7 ± 5.9% (ZnO NPs), and 38.9 ± 6.2% (control; $P < 0.05$ for all comparisons). The PRP group demonstrated the most organized collagen architecture with mature, well-aligned fibers.

Angiogenesis Assessment: Blood vessel density was significantly increased in both treatment groups, with PRP showing the highest vascular density.

Immunohistochemical Analysis

Growth Factor Expression: VEGF expression was significantly upregulated in both treatment groups, with highest expression in the PRP group. VEGF H-scores on Day 14 were: Control 127.4 ± 18.7, ZnO NPs 168.9 ± 22.1*, PRP 198.5 ± 24.8*# ($P < 0.001$ for both treatments vs. control; $P = 0.008$ for PRP vs. ZnO NPs).

TGF-β expression showed significant enhancement in treatment groups. TGF-β H-scores at Day 14 were: Control 89.7 ± 15.3, ZnO NPs 134.6 ± 19.8*, PRP 156.2 ± 21.7*# ($P < 0.001$ for both treatments vs. control; $P = 0.012$ for PRP vs. ZnO NPs).

Inflammatory Markers: TNF-α expression was significantly reduced in both treatment groups. TNF-α H-scores at Day 7 were: Control 178.9 ± 26.4, ZnO NPs 132.7 ± 21.8*, PRP 98.6 ± 18.2*# ($P < 0.001$ for both treatments vs. control; $P = 0.003$ for PRP vs. ZnO NPs).

PDGF expression was enhanced in both treatment groups. PDGF H-scores on Day 7 were: Control 95.8 ± 16.9, ZnO NPs 142.3 ± 20.4*, PRP 171.8 ± 23.6*# ($P < 0.001$ for both treatments vs. control; $P = 0.007$ for PRP vs. ZnO NPs).

Safety Assessment

No adverse events or complications were observed in any treatment group. Local tolerance was excellent for both PRP and ZnO NPs applications. No signs of hypersensitivity reactions, excessive inflammation, or delayed healing complications were noted.

Discussion

This comparative study provides compelling evidence that both autologous PRP and ZnO NPs significantly enhance cutaneous wound healing compared to standard care, with PRP demonstrating superior therapeutic efficacy across multiple assessment parameters. These findings contribute valuable insights to advanced wound healing modalities and establish direct comparison between biological and nanotechnology-based therapeutic approaches [19-21].

The superior performance of PRP aligns with extensive literature documenting its wound healing benefits. Our findings show 27% reduction in healing time and 95.6% wound closure on Day 15 are consistent with previous studies [22, 23]. The molecular basis for PRP's efficacy, particularly the significant upregulation of VEGF (H-score: 198.5 ± 24.8) and TGF-β (H-score: 156.2 ± 21.7), is well-supported by mechanistic research demonstrating that PRP contains numerous growth factors promoting wound healing and angiogenesis [5, 24].

Our immunohistochemical findings showing reduced TNF-α expression (H-score: 98.6 ± 18.2) in PRP-treated wounds indicate effective modulation of inflammatory response. The enhanced angiogenesis observed in our PRP group (51.9 ± 7.2 vessels/mm² at Day 14) is particularly noteworthy, corroborating findings regarding PRP's potent pro-angiogenic effects [25].

ZnO NPs demonstrated promising alternative therapeutic potential, achieving 16% reduction in healing time and 89.7% wound closure at Day 15. These results align with emerging literature highlighting nanotechnology-based wound treatment potential [26]. The antimicrobial properties of ZnO NPs likely contributed to favorable healing environments observed histologically. The enhanced collagen deposition in our ZnO NPs group (48.7 ± 5.9% at Day 21) reflects the material's ability to promote extracellular matrix remodeling.

The direct comparison reveals important therapeutic distinctions. While both treatments significantly outperformed controls, PRP's biological approach utilizing endogenous growth factors appeared more effective than ZnO NPs'

physicochemical mechanisms. This difference may reflect wound healing's complex multi-factorial nature, where biological signals play dominant roles over antimicrobial and physical effects alone.

The differential expression patterns in our immunohistochemical analysis provide insight into distinct therapeutic mechanisms [27, 28]. PRP's superior performance in upregulating VEGF and TGF-β while simultaneously downregulating TNF-α suggests more comprehensive modulation of wound healing cascades. This biological approach harnesses natural healing mechanisms, potentially explaining its superior efficacy [29].

Several limitations warrant consideration. The single-dose treatment protocol may not represent optimal therapeutic regimens. The fixed ZnO NPs concentration (1 mg/mL) may not represent optimal therapeutic dose. The 21-day follow-up precluded assessment of long-term outcomes and scar quality. Clinical implications extend beyond specific treatments evaluated. PRP's autologous nature eliminates immune rejection and disease transmission concerns, making it attractive for clinical application. However, blood collection and processing requirements may limit accessibility in resource-constrained settings. ZnO NPs offer advantages in standardization, shelf stability, and ease of application.

Future research directions include combination therapy approaches utilizing both treatments, optimization of treatment protocols, and clinical trials validating these experimental findings in human populations.

Conclusion

This study demonstrates that both autologous PRP and ZnO NPs significantly enhance cutaneous wound healing compared to standard care, with PRP showing superior therapeutic efficacy. The 27% reduction in healing time with PRP versus 16% with ZnO NPs, combined with superior histological and molecular outcomes, establishes PRP as more effective in our experimental model. However, both treatments showed excellent safety profiles and meaningful therapeutic benefits, supporting their potential clinical applications.

The mechanistic insights reveal distinct therapeutic pathways, with PRP demonstrating more comprehensive modulation of wound healing cascades through growth factor-mediated mechanisms. ZnO NPs, while less potent, offer practical advantages including standardization, stability, and potential for combination formulations.

These findings contribute to the evidence base supporting advanced wound healing therapies and provide guidance for clinical decision-making regarding treatment selection. Future research should focus on optimizing treatment protocols, investigating combination approaches, and conducting clinical trials to validate these experimental findings in human populations.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The study adhered to ARRIVE 2.0 reporting standards and institutional guidelines.

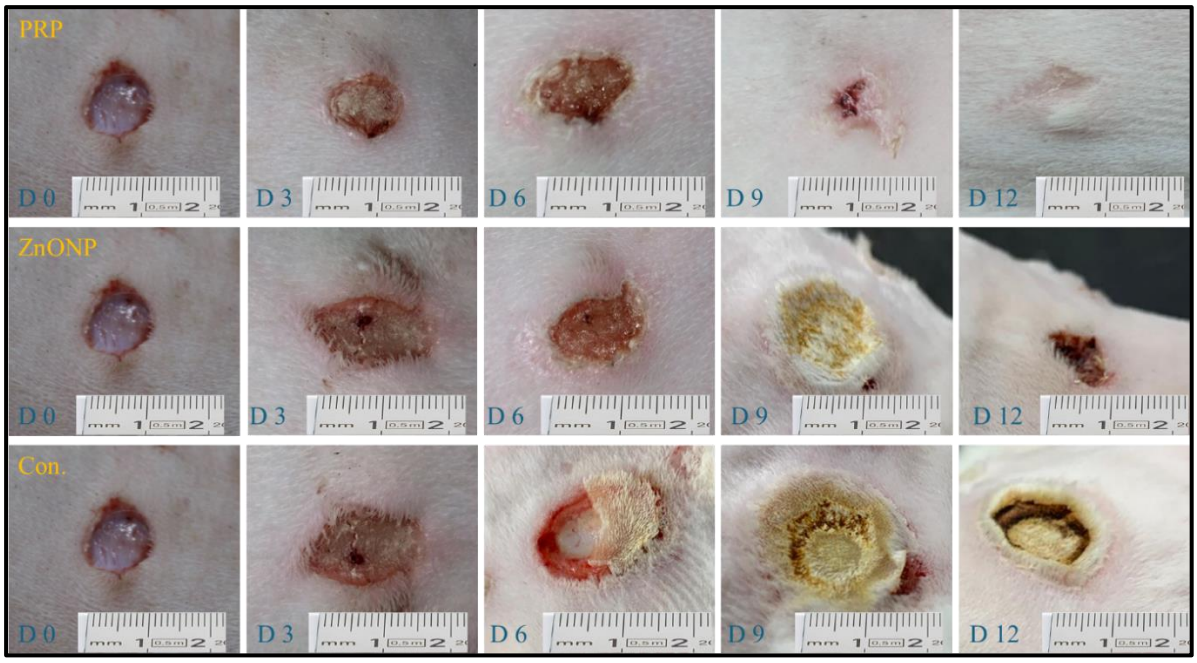

Fig. 1. Representative wound photographs showing healing progression in Control, ZnO NPs, and PRP groups during the first 12 days post-treatment. Panels are labeled accordingly.

TABLE 1. Percentage Wound Closure Over Time (%, Mean ± SD).

Time Point	Control Group	ZnO NPs Group	PRP Group	P-value
Day 3	12.8 ± 8.3	19.7 ± 9.1	27.4 ± 10.8*	0.012
Day 6	28.6 ± 11.7	42.3 ± 12.4*	55.2 ± 14.6*#	<0.001
Day 9	44.9 ± 13.8	60.1 ± 14.2*	71.3 ± 13.9*#	<0.001
Day 12	62.1 ± 15.9	76.2 ± 13.8*	84.4 ± 11.7*#	<0.001
Day 15	76.4 ± 17.2	89.7 ± 11.6*	95.6 ± 7.8*#	<0.001
Day 18	87.3 ± 14.8	96.4 ± 8.9*	99.2 ± 3.1*#	<0.001
Day 21	95.6 ± 10.1	99.6 ± 2.4*	99.9 ± 1.2*	0.031

*P < 0.05 compared to control group; #P < 0.05 compared to ZnO NPs group

TABLE 2. Histological Wound Healing Scores (Mean ± SD).

Time Point	Control Group	ZnO NPs Group	PRP Group	P-value
Day 7	4.2 ± 1.1	6.8 ± 1.3*	8.1 ± 1.2*#	<0.001
Day 14	7.1 ± 1.4	9.3 ± 1.2*	10.8 ± 1.1*#	<0.001
Day 21	9.8 ± 1.3	11.2 ± 0.9*	11.9 ± 0.8*#	<0.001

*P < 0.05 compared to control group; #P < 0.05 compared to ZnO NPs group

TABLE 3. Blood Vessel Density (vessels/mm², Mean ± SD).

Time Point	Control Group	ZnO NPs Group	PRP Group	P-value
Day 7	18.3 ± 3.7	26.8 ± 4.2*	34.5 ± 5.1*#	<0.001
Day 14	31.2 ± 5.8	42.7 ± 6.3*	51.9 ± 7.2*#	<0.001
Day 21	25.7 ± 4.9	35.4 ± 5.7*	41.8 ± 6.4*#	<0.001

*P < 0.05 compared to control group; #P < 0.05 compared to ZnO NPs group

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مقارنة فعالية البلازما الغنية بالصفائح الدموية الذاتية وجسيمات أكسيد الزنك النانوية في تسريع التئام الجروح الجلدية: دراسة عشوائية مضبوطة على الأرانب

عبد العزيز فيصل الحسين[1]، صالح عبد المنعم السيد[1]، محمد مرزوق[1]، عبد الرحمن حريبه[2]، محمد الشريف[3] ومحمد عبد القوي[3]

[1] قسم الدراسات الاكلينيكية، كلية الطب البيطري، جامعة الملك فيصل، المملكة العربية السعودية.

[2] قسم الميكروبيولوجي، كلية الطب البيطري، جامعة الملك فيصل، المملكة العربية السعودية.

[3] قسم الجراحة، كلية الطب البيطري، جامعة الوادي الجديد، مصر.

الملخص

تطلب العلاجات المتقدمة للجروح تعزيز العمليات الطبيعية للشفاء بما يتجاوز الأساليب التقليدية. هدفت هذه الدراسة إلى مقارنة فعالية البلازما الغنية بالصفائح الدموية الذاتية (PRP) مقابل جسيمات أكسيد الزنك النانوية (ZnO NPs) في التئام الجروح الجلدية الكاملة السماكة. تم توزيع ستة وثلاثين أرنبًا ذكرًا من سلالة نيوزيلندا البيضاء بشكل عشوائي إلى ثلاث مجموعات :(n=12) مجموعة معالجة بـPRP ، مجموعة معالجة بـZnO NPs ، ومجموعة ضابطة بمحلول ملحي. أُنشئت على كل حيوان جرحان دائريان بقطر 20 مم في المنطقة الظهرية. طُبّقت العلاجات يوميًا لمدة خمسة أيام، ثم يومًا بعد يوم حتى الالتئام أو حتى اليوم الحادي والعشرين. شملت المقاييس الأساسية سرعة انغلاق الجرح، الدرجات النسيجية للشفاء، والتعبير الجزيئي للواسمات الحيوية. أظهرت كلتا المعالجتين تسريعًا ملحوظًا لالتئام الجروح مقارنةً بالمجموعة الضابطة. حققت مجموعة PRP نسبة انغلاق 95.6٪ في اليوم الخامس عشر مقابل 89.7٪ لمجموعة ZnO NPs و76.4٪ للمجموعة الضابطة .(P < 0.001) تم الالتئام الكامل في 17.3 ± 2.1 يومًا (PRP) و19.8 ± 2.4 يومًا (ZnO NPs)و23.7 ± 3.2 يومًا (المجموعة الضابطة؛ .P < 0.001) أظهرت مجموعة PRP تفوقًا في الدرجات النسيجية، وزيادة في التكوّن الوعائي الدموي (51.9 ± 7.2 مقابل 42.7 ± 6.3 وعاء/مم²)، وارتفاعًا في تعبير عامل النمو البطاني الوعائي VEGF (H-score: 198.5 مقابل 168.9)، وانخفاضًا في المؤشرات الالتهابية مقارنةً بمجموعة ZnO NPs. خلصت الدراسة إلى أن كلًّا من PRP و ZnO NPsيُحسّنان بشكل كبير من التئام الجروح، مع تفوق PRP من حيث الفعالية العلاجية بفضل التعديل الشامل لمسارات الشفاء. تدعم هذه النتائج الاستخدامات السريرية لكلا العلاجين، مع تفضيل PRPللجروح المعقدة، في حين توفر ZnO NPs مزايا عملية للرعاية الروتينية.

الكلمات الدالة: البلازما الغنية بالصفائح الدموية، الجسيمات النانوية لأكسيد الزنك، التئام الجروح، التكوّن الوعائي، عوامل النمو.