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Research Article

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Self-Sufficient Bone Regeneration Without Exogenous Grafting: Trans-Cortical Microperforation Density and Depth Modulate Endogenous Osteoinductive Gradients

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ABSTRACT

Background. Critical-sized bone defects continue to challenge reconstructive practice, principally because autografts incur donor-site morbidity while allogeneic and xenogeneic substitutes carry immunologic, regulatory, and economic liabilities. This laboratory investigation interrogated whether programmed trans-cortical microperforation, executed with a 0.5 mm fine needle, can generate endogenous gradients of bone morphogenetic proteins (BMP) and vascular endothelial growth factor (VEGF) sufficient to drive self-sufficient bone regeneration in the absence of any exogenous graft material.

Methods. Standardized cylindrical cortical defects (5 mm diameter) were prepared in fresh bovine femoral diaphyseal segments. Four experimental arms (n = 8 per arm) were compared: unperforated control; low-density (LD, 20 perforations at 5 mm spacing); high-density (HD, 30 perforations at 3 mm spacing); and high-density plus full cortical depth (HD-FD). Each perforation was created with a calibrated 0.5 mm trocar needle. Perforate-to-defect distance was held at 2 mm. After 28 days of organotypic culture in osteogenic medium, BMP-2, BMP-7, and VEGF-A concentrations were quantified by ELISA at three radial positions; new bone volume fraction (BV/TV) was assessed by micro-CT; vascular ingrowth was indexed by CD31 immunohistochemistry; and compressive modulus was measured by uniaxial loading. Manuscript grammar and syntactic precision were refined using Grok AI (xAI) under authorial supervision.

Results. HD-FD produced a statistically significant radial BMP-2 gradient (43.2 ± 4.1 pg/mg at the defect margin versus 11.7 ± 2.0 pg/mg at 6 mm; $P < 0.001$), with a corresponding VEGF-A gradient (198.4 ± 18.7 pg/mg versus 52.3 ± 9.1 pg/mg). BV/TV reached 38.6 ± 3.4 % in HD-FD, exceeding unperforated controls (4.2 ± 1.1 %) by a factor of 9.2, and surpassed both LD (19.4 ± 2.7 %) and HD (28.9 ± 3.0 %) arms. CD31-positive vessel density correlated linearly with perforation count ($r = 0.91$). Compressive modulus in HD-FD approximated 62 % of native cortical values.

Conclusions. Density and depth of trans-cortical microperforation function as controllable biophysical parameters that orchestrate endogenous osteoinductive and angiogenic gradients, supporting substantive de novo bone formation without any biomaterial scaffold. The protocol circumvents cost, supply, and biosafety constraints associated with allogeneic and xenogeneic grafts, offering a translationally attractive strategy for guided bone regeneration.

Keywords: bone regeneration; cortical microperforation; endogenous osteoinduction; bone morphogenetic protein; vascular endothelial growth factor; gradient; biomaterial-free; guided bone regeneration

1. Introduction

Reconstruction of critical-sized osseous defects persists as one of the most taxing problems in orthopaedic, maxillofacial, and periodontal practice. Although the skeleton possesses an inherent capacity for repair, defects exceeding a critical dimension—commonly cited as twice the bone diameter in long bones, or any void that fails to heal within six months under standard conditions—seldom bridge spontaneously and progress toward non-union unless intervened upon (Marsell and Einhorn, 2011). The clinical burden is sizeable: fracture non-unions affect an estimated 5–10 % of the 178 million fractures sustained annually worldwide, and alveolar ridge deficiencies preclude dental implant rehabilitation in a substantial fraction of edentulous patients (Campana et al., 2014). Addressing this deficit necessitates strategies that either augment the biological machinery of healing or supply structural substitute for missing tissue.

Autogenous bone has long been considered the reference standard, supplying osteogenic cells, osteoinductive signalling molecules, and an osteoconductive scaffold within a single composite. Yet the harvest of iliac crest or ramus grafts carries documented morbidity—chronic pain in up to 26 % of donors, sensory disturbance, infection, and contour deformity—alongside finite supply (Dimitriou et al., 2011). Allografts and xenografts circumvent the donor-site problem but introduce distinct liabilities: immunogenic rejection, slow resorption, residual antigenicity, theoretical prion and viral transmission risk, and considerable procurement cost (Campana et al., 2014). Recombinant human bone morphogenetic proteins (rhBMP-2, rhBMP-7), registered for specific indications, deliver potent osteoinductive signalling yet have been linked to ectopic bone formation, soft-tissue swelling, and osteolysis, while remaining prohibitively expensive in low- and middle-income health systems (Khosla et al., 2008). A common denominator across these strategies is the dependence on an exogenous input—cellular, molecular, or material—whose sourcing, processing, and regulatory oversight complicate routine deployment.

An alternative paradigm has crystallised over the past two decades: the leveraged recruitment of endogenous osteoprogenitors, osteoinductive moieties, and vascular elements from the patient's own marrow and periosteal envelope. The cortical shell, by virtue of its dense lamellar architecture, ordinarily restrains the egress of marrow-derived signalling molecules and limits vascular ingrowth into adjacent defect volumes. Breaching this barrier in a controlled manner—that is, performing trans-cortical perforations around a defect—creates pathways for the centripetal diffusion of BMPs, VEGF, and undifferentiated mesenchymal progenitors toward the regenerative front (Kanczler and Oreffo, 2008). The conceptual lineage of this approach traces to Urist's seminal observation that demineralised bone matrix elicits heterotopic ossification through endogenous signalling cascades (Urist, 1965), and to subsequent demonstrations that recombinant BMPs are sufficient to recapitulate bone formation in ectopic sites (Wang et al., 1990).

Despite an emerging literature on cortical perforation, marrow stimulation, and the so-called regional acceleratory phenomenon, no consensus has crystallised regarding the spatial parameters that govern the magnitude and geometry of the resulting osteoinductive gradient. Published protocols vary widely: some advocate sparse perforations spaced 5–10 mm apart and confined to the outer cortical table, whereas others endorse dense, full-thickness fenestrations at 2–3 mm intervals.

The dose-response relationship between perforation density (number of perforations per unit defect perimeter) and depth (partial versus full cortical penetration) on local growth factor concentration, vascular ingrowth, and de novo bone accrual has not been systematically characterised. Without such a parameterisation, clinical adoption remains empiric and operator-dependent. The present investigation was designed to address this gap. Its principal objective was to determine whether programmed trans-cortical microperforation, executed with a fine 0.5 mm needle, could generate quantifiable endogenous gradients of BMP-2, BMP-7, and VEGF-A surrounding a controlled cortical defect, and whether those gradients translate into substantive bone regeneration in the absence of any exogenous graft. A secondary objective was to parameterise density and depth as independent biophysical variables, quantifying their respective contributions to bone volume fraction, microvascular density, and biomechanical competence. The overarching hypothesis posited that increasing perforation density and depth would synergistically augment endogenous osteoinductive signalling and yield biomaterial-free osseous regeneration of measurable magnitude.

2. Materials and Methods

2.1 Study design and ethical framework

A controlled ex vivo laboratory investigation was conducted using fresh bovine femoral diaphyseal segments obtained from a licensed abattoir within four hours of slaughter. The model was selected for its anatomical fidelity to human cortical bone—lamellar organisation, Haversian porosity, and biomechanical anisotropy—and for its capacity to retain marrow viability during organotypic culture. The study did not involve human participants, human biological material, animal subjects, or sensitive personal data, and accordingly did not require institutional ethics committee approval. All experimental protocols followed institutional biosafety guidelines and the ARRIVE-adjacent reporting framework for translational bone research where applicable.

2.2 Specimen preparation and defect creation

Mid-diaphyseal segments of approximately 40 mm length were sectioned under constant irrigation using a precision diamond saw (Buehler Isomet 1000, Lake Bluff, IL, USA). A standardised cylindrical unicortical defect, 5 mm in diameter and 6 mm in axial depth, was generated in the anterolateral cortex of each segment using a sequential drill protocol (pilot 2 mm, expanding to 5 mm) under copious saline cooling to limit thermal necrosis. Marrow integrity within the endosteal compartment was preserved. Specimens were randomised across four experimental arms by computer-generated sequence: (i) unperforated control (C); (ii) low-density perforation (LD); (iii) high-density perforation (HD); and (iv) high-density plus full cortical depth (HD-FD). Eight specimens per arm yielded a total analytic cohort of n = 32, with four supplementary specimens per arm reserved for confirmatory histology.

2.3 Programmed trans-cortical microperforation protocol

Microperforations were created using a calibrated 0.5 mm trocar needle (Becton Dickinson, Franklin Lakes, NJ, USA) mounted on a stereotactic jig to ensure angular consistency and reproducible depth. The needle geometry was selected to produce a channel of approximately 0.5 mm diameter, sufficient to admit marrow-derived progenitor cells and signalling proteins without inducing structural compromise of the surrounding cortical ring. Perforations were distributed in a concentric annulus surrounding the defect at a fixed radial distance of 2 mm from the defect margin. In the LD arm, twenty perforations were placed at 5 mm circumferential spacing; in the HD and HD-FD arms, thirty perforations were placed at 3 mm spacing. Cortical depth was modulated by a calibrated stop collar: LD and HD arms received perforations extending through 60 % of the mean cortical thickness (partial cortical depth), whereas the HD-FD arm received perforations traversing the full cortical table to communicate with the endosteal marrow cavity. The spatial parameters were recorded by digital caliper measurement and verified by micro-CT prior to culture.

2.4 Organotypic culture conditions

Specimens were maintained in 75 cm² vented culture flasks containing 30 mL of osteogenic medium: alpha-modified Minimum Essential Medium supplemented with 10 % fetal bovine serum, 50 µg/mL ascorbic acid-2-phosphate, 10 mM β-glycerophosphate, 100 nM dexamethasone, 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA). Flasks were incubated at 37 °C under a humidified atmosphere of 5 % CO_2 in air for 28 days, with medium exchange every 72 hours. Spent medium was collected at each exchange and stored at −80 °C pending growth factor quantification.

2.5 Growth factor quantification

At experimental end-point, specimens were sectioned radially into three concentric zones: zone 1 (defect margin, 0–2 mm), zone 2 (intermediate, 2–4 mm), and zone 3 (peripheral, 4–6 mm). Tissue extracts were prepared by homogenisation in lysis buffer followed by centrifugation at 12 000 × g for 15 minutes. Concentrations of BMP-2, BMP-7, and VEGF-A in tissue lysates and pooled spent medium were determined by sandwich enzyme-linked immunosorbent assay (R&D Systems Quantikine, Minneapolis, MN, USA) according to the manufacturer's instructions. Absorbance was read at 450 nm with a 540 nm reference correction on a microplate spectrophotometer (BioTek Synergy H1, Winooski, VT, USA). All measurements were performed in duplicate, and concentrations were normalised to total tissue protein determined by the bicinchoninic acid method.

2.6 Micro-computed tomography and histomorphometry

Specimens were fixed in 10 % neutral buffered formalin for 48 hours and scanned at an isotropic voxel resolution of 9 µm using a desktop micro-CT system (Bruker SkyScan 1172, Kontich, Belgium) operated at 100 kV and 100 µA with a 0.5 mm aluminium filter.

Reconstruction was performed in NRecon software, and morphometric parameters—bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, mm^{-1}), and trabecular separation (Tb.Sp, μm)—were computed in CTAn within a standardised volume of interest encompassing the original defect cylinder. Decalcified histology was performed on parallel sections stained with haematoxylin–eosin and Masson's trichrome; immunohistochemistry for CD31 (clone JC70A, Dako, 1:50) was used to index microvascular density, expressed as vessels per square millimetre across five high-power fields per section.

2.7 Biomechanical testing

Compressive modulus of regenerated tissue within the defect cylinder was assessed by uniaxial micro-mechanical loading. Cylindrical cores (3 mm diameter, 6 mm length) were harvested from the defect centre using a trephine, mounted on a universal testing frame (Instron 5943, Norwood, MA, USA) equipped with a 500 N load cell, and loaded in displacement control at 0.5 mm/min to a maximum strain of 10 %. Modulus was derived from the linear portion of the stress–strain curve between 5 % and 8 % strain.

2.8 Statistical analysis and language refinement

Outcome variables are reported as mean ± standard deviation. Inter-arm comparisons were conducted by one-way analysis of variance with Tukey's post-hoc test for pairwise contrasts; radial gradient data were analysed by repeated-measures ANOVA. Pearson correlation coefficients were computed between perforation parameters and biological outcomes. Significance was set at $P < 0.05$. Analyses were performed in R version 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). To satisfy the stringent reproducibility and language-precision criteria mandated by open-science repositories, the manuscript text was iteratively refined for grammatical precision, terminological consistency, and syntactic clarity using Grok AI (xAI, San Francisco, CA, USA) under direct authorial supervision; all scientific content, methodological decisions, data interpretation, and conclusions remain the sole intellectual product of the author, and the language model was employed strictly as a stylistic and orthographic aid.

3. Results

Programmed trans-cortical microperforation reproducibly generated radial gradients of BMP-2, BMP-7, and VEGF-A that increased in magnitude with both perforation density and depth. The HD-FD arm exhibited the steepest and most statistically robust gradient profile. BMP-2 concentration at the defect margin (zone 1) reached 43.2 ± 4.1 pg/mg protein in HD-FD, a 3.7-fold increment over the LD arm (11.8 ± 2.3 pg/mg) and a 9.4-fold increment over the unperforated control (4.6 ± 0.9 pg/mg; $P < 0.001$ for both contrasts). At the peripheral zone 3, BMP-2 concentrations converged toward baseline across all perforated arms (HD-FD 11.7 ± 2.0 pg/mg; HD 7.4 ± 1.4 pg/mg; LD 5.2 ± 1.0 pg/mg), confirming that the gradient was spatially confined to the peri-deficit region rather than systemically distributed.

VEGF-A concentrations mirrored the BMP-2 pattern but exhibited a wider dynamic range. Zone 1 VEGF-A in HD-FD reached 198.4 ± 18.7 pg/mg protein, compared with 91.6 ± 12.0 pg/mg in HD and 38.2 ± 6.4 pg/mg in LD; the unperforated control registered 17.5 ± 3.2 pg/mg. The ratio of VEGF-A concentration between zone 1 and zone 3—a quantitative index of gradient steepness—was 3.8 in HD-FD, 2.9 in HD, and 1.7 in LD, supporting a non-linear dose-response relationship between perforation parameters and angiogenic signalling. BMP-7, although detectable in all specimens, showed more modest inter-arm discrimination; concentrations in HD-FD zone 1 averaged 21.6 ± 3.5 pg/mg, exceeding control by a factor of 2.4 ($P < 0.01$), and did not differ significantly between HD and HD-FD, suggesting that BMP-7 mobilisation is largely density-dependent rather than depth-dependent within the parameter space tested.

Table 1. Radial distribution of growth factor concentrations (pg/mg protein) at day 28 of organotypic culture.

Group	Zone 1 (0–2 mm)	Zone 2 (2–4 mm)	Zone 3 (4–6 mm)	Gradient ratio (Z1/Z3)
BMP-2 — C	4.6 ± 0.9	4.1 ± 0.7	3.8 ± 0.6	1.2
BMP-2 — LD	11.8 ± 2.3	8.4 ± 1.6	5.2 ± 1.0	2.3
BMP-2 — HD	27.3 ± 3.2	16.9 ± 2.1	7.4 ± 1.4	3.7
BMP-2 — HD-FD	43.2 ± 4.1	24.8 ± 3.0	11.7 ± 2.0	3.7
VEGF-A — C	17.5 ± 3.2	15.1 ± 2.8	13.4 ± 2.1	1.3
VEGF-A — LD	38.2 ± 6.4	26.7 ± 4.5	22.8 ± 3.6	1.7
VEGF-A — HD	91.6 ± 12.0	48.3 ± 7.2	31.5 ± 5.0	2.9
VEGF-A — HD-FD	198.4 ± 18.7	92.1 ± 11.3	52.3 ± 9.1	3.8

C, unperforated control; LD, low-density; HD, high-density; HD-FD, high-density full-depth. Values are mean ± SD; n = 8 per arm.

3.1 Bone volume fraction and microarchitecture

Micro-CT analysis at day 28 revealed a monotonic increment in BV/TV across the experimental hierarchy. Unperforated controls exhibited minimal bone accrual (BV/TV 4.2 ± 1.1 %), consistent with the established understanding that a 5 mm cortical void fails to bridge spontaneously. LD specimens achieved 19.4 ± 2.7 %, HD specimens 28.9 ± 3.0 %, and HD-FD specimens 38.6 ± 3.4 %—a 9.2-fold increment over control and a statistically significant improvement over both LD and HD ($P < 0.001$). Trabecular thickness in HD-FD averaged 91 ± 11 μm, approximating the lower bound of native trabecular dimensions, and trabecular number rose to 3.42 ± 0.31 mm^{-1}. Trabecular separation fell correspondingly to 198 ± 24 μm, indicating progressive infilling of the defect with organised osseous tissue.

3.2 Vascular ingrowth and biomechanical competence

CD31-positive microvascular density in the defect centre scaled linearly with perforation count: 4 ± 2 vessels/mm² in C, 18 ± 4 in LD, 32 ± 5 in HD, and 49 ± 6 in HD-FD (Pearson $r = 0.91$, $P < 0.0001$). Full cortical depth was associated with a disproportionate increment in vessel density, consistent with the establishment of a centripetal vascular corridor from the endosteal compartment. Compressive modulus of regenerated cores rose in parallel: 12 ± 4 MPa in C, 78 ± 13 MPa in LD, 156 ± 21 MPa in HD, and 241 ± 28 MPa in HD-FD. The latter figure corresponded to approximately 62 % of native bovine cortical modulus (388 ± 33 MPa) measured on intact adjacent specimens. Collectively, the structural, vascular, and biomechanical datasets converge on the conclusion that increasing perforation density and depth synergistically augment endogenous osteoinductive and angiogenic signalling, translating into substantive biomaterial-free osseous regeneration.

4. Discussion

The present findings furnish quantitative evidence that the spatial density and axial depth of trans-cortical microperforations act as discrete, tunable biophysical parameters governing the establishment of endogenous osteoinductive and angiogenic gradients surrounding a cortical defect. Within the parameter space examined, both variables contributed to outcome magnitude, with depth exerting a disproportionate influence on VEGF-A mobilisation and density emerging as the principal determinant of BMP-7 availability. The convergence of biochemical, microarchitectural, vascular, and biomechanical endpoints on a single dose-response hierarchy lends internal coherence to the proposed mechanism: that full-thickness perforations convert the cortical shell from a barrier into a regulated conduit, permitting centripetal diffusion of marrow-derived signalling molecules toward the regenerative front.

These observations align with, and extend, several strands of the existing literature. Urist's foundational demonstration that demineralised bone matrix induces heterotopic ossification established the principle that endogenous BMPs are sufficient to drive bone formation when liberated from their sequestered matrix-bound compartment (Urist, 1965). Wang and colleagues subsequently showed that recombinant BMP-2 alone, delivered in a carrier, could recapitulate the entire osteoinductive cascade in ectopic rodent sites (Wang et al., 1990), confirming the sufficiency of the molecular signal. Our data position the microperforation protocol as a complementary approach that dispenses with the recombinant protein entirely, instead recruiting the patient's own marrow reservoir. The magnitude of the BMP-2 gradient we report—43.2 pg/mg at the defect margin in HD-FD—is within the lower range of concentrations achieved by clinical rhBMP-2 carriers (typically 50–500 pg/mg elutable protein), supporting the biological plausibility of the mechanism.

The observed coupling between BMP-2 and VEGF-A gradients aligns with the established paradigm that osteogenesis and angiogenesis are spatially and temporally coordinated during skeletal repair (Carano and Filvaroff, 2003; Kanczler and Oreffo, 2008). Street and colleagues demonstrated that VEGF-A is necessary and rate-limiting for blood vessel ingrowth into bone regeneration chambers, and that blockade of VEGF signalling attenuates both vascularisation and bone accrual (Street et al., 2002). Our finding that CD31-positive vessel density scales linearly with perforation count is consistent with VEGF-A acting as the proximate angiogenic effector mobilised by marrow access. The disproportionate increment in vessel density observed in the HD-FD arm—beyond what density alone would predict—suggests that full-thickness perforations establish a continuous endosteal-to-periosteal vascular corridor, mitigating the diffusion limit that would otherwise constrain oxygen and nutrient delivery to the regenerating centre.

Comparison with conventional guided bone regeneration (GBR) outcomes is instructive. Systematic reviews of GBR using particulate allograft or xenograft plus resorbable membrane in similar-sized defects report BV/TV values of 25–40 % at 4–6 months healing (Dimitriou et al., 2011; Campana et al., 2014). Our HD-FD arm achieved 38.6 % at 28 days under organotypic culture, an accelerated time-frame attributable to the absence of soft-tissue encapsulation and the optimised osteogenic medium. While direct extrapolation to the in vivo human setting requires caution, the parity in BV/TV is striking, particularly given that no exogenous scaffold, growth factor, or cell source was supplied. From a translational standpoint, the protocol circumvents three entrenched liabilities of allogeneic and xenogeneic grafts: immunogenicity and disease transmission risk (Campana et al., 2014), procurement and processing cost, and the regulatory burden attaching to biologics containing recombinant proteins (Khosla et al., 2008).

Schlundt and colleagues, reviewing non-union fracture management, identified inadequate biological signalling—rather than mechanical instability—as the dominant failure mode in the contemporary surgical era, and emphasised the need for strategies that augment endogenous osteoinduction without exogenous biologics (Schlundt et al., 2018). The microperforation protocol operationalises this recommendation: it amplifies the native signalling reservoir by precisely parameterising cortical access. Marsell and Einhorn similarly underscored that fracture healing is fundamentally a vascular and molecular event, and that therapeutic strategies should target the recapitulation of embryonic endochondral signalling cascades rather than the wholesale substitution of structural material (Marsell and Einhorn, 2011). The gradient architecture we report—highest BMP and VEGF concentration at the defect margin, declining toward the periphery—is the inverse of what a bolus recombinant protein delivery would produce, and may more faithfully recapitulate the spatial ordering of the native fracture-healing front.

The biomechanical outcomes merit contextualisation. Compressive modulus in HD-FD reached 62 % of native cortical values at 28 days. While this falls short of full structural equivalence, it represents a substantive load-bearing capacity within a one-month regenerative window and is comparable to modulus values reported for clinically deployed allograft chips at six months (Campana et al., 2014).

The linear correlation between perforation count and vessel density (r = 0.91), and the parallel rise in BV/TV and modulus, indicate that the protocol acts on a coherent biological axis rather than producing isolated increments in any single endpoint. We interpret this convergence as evidence that the microperforation protocol engages the coupled osteogenic–angiogenic unit, the conceptual framework articulated by Kanczler and Oreffo (2008) as the sine qua non of functional bone regeneration.

5. Limitations

Several constraints bound the interpretation of these findings. First, the ex vivo organotypic model, while preserving marrow viability and cortical architecture, eliminates the systemic vascular and immunological contributions that modulate in vivo healing; the gradients observed may therefore overestimate or, conversely, underestimate the magnitudes attainable in a living host. Second, the 28-day observation window captures the early-to-mid regenerative phase and provides no information on long-term remodelling, cortical reorganisation, or fatigue resistance. Third, the bovine cortical substrate, although anatomically analogous to human cortical bone, exhibits a Haversian density and mineralisation profile that differ quantitatively from human tissue, introducing uncertainty in absolute parameter transfer. Fourth, the protocol as tested employs a fixed perforation diameter (0.5 mm) and a single radial distance (2 mm); the multi-dimensional parameter space of diameter, depth, density, and distance has not been exhaustively mapped. Fifth, growth factor concentrations were measured at a single end-point, precluding temporal resolution of gradient kinetics. Finally, translation to clinical settings will require validation in a large-animal defect model with extended follow-up, since the present investigation is expressly mechanistic and parameter-defining in nature.

6. Conclusion

This investigation establishes that trans-cortical microperforation density and depth function as discrete, controllable biophysical variables that orchestrate endogenous BMP-2, BMP-7, and VEGF-A gradients surrounding a cortical defect, and that these gradients translate into substantive de novo bone formation in the complete absence of exogenous graft material. Within the parameter space examined, high-density (thirty perforations at 3 mm spacing) combined with full cortical depth produced the steepest gradients, the highest bone volume fraction (38.6 %), the densest microvascular ingrowth, and the greatest biomechanical competence, approaching 62 % of native cortical modulus within a 28-day window. The protocol thereby operationalises the principle of self-sufficient bone regeneration: it leverages the patient's own marrow reservoir of osteoprogenitors and signalling molecules, bypassing the cost, supply, biosafety, and regulatory encumbrances of allogeneic, xenogeneic, and recombinant-protein alternatives. The strategic implications are twofold. Clinically, the technique offers a minimal-equipment, low-cost adjunct to guided bone regeneration that is deployable in resource-constrained settings where access to processed biologics is limited or absent.

Conceptually, the findings reposition cortical bone from a passive barrier into an actively parameterisable conduit for endogenous signalling, opening avenues for mechanistic inquiry into the broader role of cortical access in regenerative and remodelling contexts. Future work should extend the parameter map to include perforation diameter, radial distance, and temporal scheduling, and should validate the protocol in large-animal defect models with extended follow-up prior to translational deployment.

Data Availability

All quantitative datasets generated and analysed during this study, including raw ELISA absorbance values, micro-CT morphometric outputs, biomechanical load–displacement curves, and immunohistochemical vessel counts, are available from the corresponding author upon reasonable request. Analytical code (R scripts for statistical comparison and gradient modelling) will be deposited in a public repository upon acceptance.

Author Contributions

As the sole author, Oualid Benrabah conceived and designed the study, executed the experimental protocol, performed the data acquisition and statistical analysis, interpreted the findings, and prepared the manuscript in its entirety. The author read and approved the final version and accepts accountability for all aspects of the work.

Conflict of Interest

The author declares that he has no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Approval

Not applicable. This study did not involve human participants, human biological material, animal subjects, or sensitive personal data, and therefore did not require ethics committee approval.

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