

EGGSHELL-DERIVED HYDROXYAPATITE COMBINED WITH NANO CALCIUM SULFATE AND PRF AS AUTOLOGOUS BIOLOGICS AND SUSTAINABLE BIOMINERALS FOR MAXILLOFACIAL REGENERATION - A NARRATIVE REVIEW

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ABSTRACT

Maxillofacial bone defects present a complex reconstructive challenge due to the intricate anatomy and aesthetic requirements of the craniofacial region. Conventional grafting methods, including autografts and allografts, are effective but constrained by donor site morbidity, immune responses, and limited availability. Recent advancements in biomaterials have led to the development of sustainable and bioactive alternatives such as eggshell-derived hydroxyapatite (EnHA), nano calcium sulfate (nCaSO₄), and platelet-rich fibrin (PRF). This narrative review synthesizes current evidence from 2019 to 2025 on the combined use of EnHA, nCaSO₄, and PRF in bone regeneration within the maxillofacial region. A systematic literature search of PubMed, ScienceDirect, and Google Scholar identified *in vitro*, *in vivo*, and clinical studies investigating these biomaterials. Findings indicate that EnHA acts as an osteoconductive scaffold supporting bone cell attachment and mineral deposition, while nCaSO₄ provides ionic calcium and a resorbable matrix that promotes early mineralization. PRF contributes autologous growth factors that enhance angiogenesis, osteoblast differentiation, and tissue maturation. The interaction among these components fosters balanced bone formation, resorption, and vascularization, leading to improved graft integration and accelerated healing. This combination represents a cost-effective, biocompatible, and environmentally sustainable alternative to conventional bone grafts. Further standardized preclinical and clinical studies are necessary to optimize formulation parameters and validate long-term outcomes for predictable maxillofacial bone regeneration.

KEYWORDS: Eggshell-derived hydroxyapatite; nano calcium sulfate; platelet-rich fibrin; maxillofacial bone regeneration; sustainable biomaterials.

INTRODUCTION

Maxillofacial bone defects resulting from traumatic injury, tooth extraction, infection, or limited surgical resections pose substantial functional and aesthetic challenges and frequently compromise subsequent rehabilitation such as dental implant placement. Conventional options including autografts, allografts, and xenografts remain widely used, but each carries important limitations: donor-site morbidity and limited supply for autografts, and cost, variable incorporation and immunologic or disease-transmission concerns for allografts/xenografts. These constraints have driven interest in alternative, cost-effective biomaterials that can match the biological and mechanical needs of facial bone reconstruction while minimizing patient morbidity. Recent reviews and pilot studies underline the increasing clinical interest in locally-sourced, engineered biomaterials as feasible alternatives for small to moderate maxillofacial defects.^[1]

Eggshell-derived nano-hydroxyapatite (EnHA) has emerged as a promising osteoconductive substitute because eggshell is abundant, low-cost, and can be processed into a biocompatible calcium-phosphate phase with a porous microstructure favorable for cell attachment. In parallel, nano calcium sulfate (nCaSO_4) offers a rapidly resorbable scaffold that provides early mechanical support and releases calcium ions that may promote early mineral nucleation and osteoblastic activity. Preclinical and pilot clinical reports indicate that EnHA and CaSO_4 that applied singly or as complementary coatings can improve bone fill and mechanical integration around implants and in extraction sockets. Together these inorganic components provide a mineral framework that supports new bone formation while being amenable to formulation and sterilization for clinical use.^[2]

Platelet-Rich Fibrin (PRF) functions as an autologous biologic adjunct that supplies a fibrin matrix rich in platelets, leukocytes and a sustained reservoir of growth factors (e.g. PDGF, TGF- β , VEGF) that accelerate angiogenesis, cellular recruitment and osteogenic differentiation. Clinical trials and case series using PRF as a membrane or incorporated into “sticky bone” composites consistently show improved soft-tissue healing, earlier revascularization and increased radiographic bone density when combined with particulate grafts. Notably, several recent clinical pilot studies that combined eggshell-derived HA or synthetic grafts with PRF reported superior volumetric and density outcomes relative to controls, supporting PRF’s role in enhancing graft incorporation and early remodeling.^[3,4]

The theoretical and early empirical rationale therefore supports a tripartite approach EnHA for osteoconduction, nCaSO_4 for resorbable structural support and ionic stimulation, and PRF for biological activation and vascularization to treat minor maxillofacial bone defects. Nevertheless, extant studies are largely small, heterogeneous in materials and protocols, and short in follow-up, leaving important gaps about optimal material ratios, degradation timing, standardized PRF preparation and long-term functional outcomes. To justify broader clinical adoption and publication in high-impact venues, rigorous, standardized trials and well-designed preclinical studies are required that quantify volumetric bone fill, bone mineral density, histomorphometry and clinical endpoints such as implant stability and complication rates. This manuscript therefore synthesizes recent experimental and clinical evidence and proposes a rationale for standardized evaluation of the EnHA + nCaSO_4 + PRF composite in maxillofacial bone grafting.

METHODS

A systematic literature search was performed in PubMed, ScienceDirect, and Google Scholar to identify studies published between 2019 and 2025 using the Boolean strategy: (“eggshell” OR “egg shell” OR “egg membrane”) AND (“nano calcium sulfate” OR “calcium sulfate”) AND (“platelet-rich fibrin” OR “PRF”) AND (“bone grafting” OR “bone regeneration”) AND (“maxillofacial” OR “facial”) AND (“bone defects” OR “defects”). Titles and abstracts were

screened for relevance to the combined application of eggshell-derived materials, nano calcium sulfate, and platelet-rich fibrin in maxillofacial bone regeneration. Eligible full-text articles including *in vitro*, *in vivo*, and clinical studies were retrieved and evaluated in detail. Studies were included if they investigated any combination of these materials for bone defect repair and reported quantitative or histological outcomes. Data extraction included study design, material composition, model type, and main results related to bone healing and regeneration. The findings were synthesized narratively due to heterogeneity in study methodologies, material formulations, and evaluation parameters.

RESULT AND DISCUSSION

Table 1: Key Methodological Studies in Eggshell-Derived Hydroxyapatite, Nano Calcium Sulfate and PRF in Maxillofacial Regeneration.

Authors (Year)	Methods	Country	Main result
Opriș H., <i>et al.</i> (2023) ^[5]	<i>In vivo</i> implantation of eggshell membrane (subcutaneous & intramuscular pockets) in Wistar rat.	Romania	Eggshell membrane (ESM) showed moderate cellular response, slow degradation; later encapsulation by collagen and multinucleated giant cells (foreign-body type) → biocompatible but limited as GBR membrane unless crosslinked/processed further.
Opriș H., <i>et al.</i> (2020) ^[6]	Systematic review of preclinical <i>in-vivo</i> studies using eggshell-derived materials (rodent/rabbit calvarial models, various graft forms)	Romania	Eggshell-derived biomaterials are generally biocompatible and osteoconductive with new bone formation in many animal models; studies heterogeneous in methods → need standarisasi dan studi lanjutan.
Liu Z., <i>et al.</i> (2017) ^[7]	<i>In vitro</i> MSC/BMP2 + PRP assays + <i>in vivo</i> rat critical-sized (8 mm) calvarial defect; nCS scaffold ± PRP ± BMP2-MSCs; micro-CT, histology, ALP, ARS.	USA / China collaborators	nCS + PRP + BMP2-MSCs gave significantly greater bone formation (BV/TV, ALP, calcium deposition) vs controls; PRP enhanced proliferation and osteogenic differentiation — supports synergy PRP + nCS ± growth signals.
Kadhim D.R., Hamad T.I., Fatalla A.A. (2022) ^[2]	Implant model: eggshell powder as graft around titanium implants coated with nano-CaSO ₄ ; histology & mechanical testing (implant torque)	Iraq	Combination eggshell powder + nano-CaSO ₄ coating improved peri-implant bone regeneration and implant removal torque vs unfilled controls.

Bone regeneration outcomes using the eggshell-derived nano-hydroxyapatite (EnHA) combined with nano calcium sulfate (nCaSO₄) and platelet-rich fibrin (PRF) have been encouraging in recent pilot and preclinical reports. Clinical pilot trials of socket preservation have reported statistically greater radiographic bone density and favorable trabecular patterns at 8–12 weeks when EnHA+PRF was used versus traditional DFDBA controls, and implant studies noted increased removal torque when eggshell powder was applied around nCaSO₄-coated screws.^[8,9] Quantitatively, several studies documented volumetric bone fill improvements in the range of ~15–35% over baseline within 3 months for EnHA or CaSO₄ combinations with PRF, though heterogeneity in measurement methods (CBCT HU, micro-CT, histomorphometry) complicates pooled averages.^[10] The uploaded review and dataset synthesize these findings and emphasize consistent short-term gains in bone density and mechanical stability across small cohorts and animal models. Nevertheless, most studies remain small, with variable PRF protocols and limited long-term (>12 months) histologic endpoints, so effect sizes must be interpreted cautiously. Overall, the empirical data justify advancing to larger randomized trials with standardized imaging and histomorphometric endpoints.^[7,9]

At the microscopic level the earliest stage after graft placement is the inflammatory phase, dominated by innate immune cells that determine downstream healing quality. Neutrophils and monocytes arrive within hours, with

monocytes differentiating into macrophages that adopt either a pro-inflammatory M1 phenotype or a reparative M2 phenotype; M2 polarization correlates with scaffold integration and osteogenesis. Lymphocytes and plasma cells are present in the subacute phase: plasma cells produce antibodies that may mark a mild allo/foreign-body recognition, and their density in biopsy specimens has been associated with low-grade chronic inflammation when non-biocompatible particles persist. Importantly, biomaterial surface chemistry and topography (e.g., nanoscale roughness of EnHA versus smooth synthetic beads) strongly influence macrophage polarization and foreign body giant cell (FBGC) formation, with sol-gel or nanostructured surfaces favoring M2 responses in experimental models. PRF modulates this immune environment by delivering leukocytes and anti-inflammatory cytokines in a fibrin matrix, which both recruits reparative cells and reduces prolonged neutrophilic activity, thereby limiting fibrotic encapsulation. Thus, controlling the early inflammatory milieu (minimizing persistent antigenic particles, promoting M2 phenotype) is critical to prevent plasma-cell-rich chronic inflammation that would otherwise impair osteogenesis.^[8,10]

During the proliferative/repair phase mesenchymal stromal cells (MSCs) migrate into the graft and differentiate into osteoprogenitors under the influence of local biochemical cues. PRF releases PDGF, TGF- β and VEGF in a sustained fashion, which stimulates MSC chemotaxis and proliferation and promotes angiogenesis, an essential precondition for robust osteoid deposition. Concurrently, Ca^{2+} ions liberated from nCaSO_4 increase local supersaturation and act as a nucleation stimulus for apatite crystallization, accelerating early mineral deposition and providing ionic signals that upregulate osteogenic transcription factors such as Runx2 and Osterix. On a cellular signaling level, PRF-derived growth factors activate Smad, PI3K/Akt and ERK pathways in MSCs and pre-osteoblasts, enhancing alkaline phosphatase (ALP) activity and collagen matrix synthesis steps measurable by increases in ALP and osteocalcin expression in vitro and by higher bone volume fraction in vivo. EnHA contributes a biomimetic surface with high surface area and protein-adsorbing capacity, concentrating serum proteins and BMPs that further bias MSCs toward an osteoblastic lineage. The synergy of a mineral template (EnHA), ionic stimulation (nCaSO_4) and biological activation (PRF) therefore produces faster and denser early bone formation than each component alone, as supported by histomorphometric comparisons in animal and pilot human studies.^[11,12]



Figure 1: Histologic aspect (20X, Trichrome Masson staining): subcutaneous tissue sample 48 hours after implantation, showing the easily visible shell membrane extending towards the deep muscle.^[5]

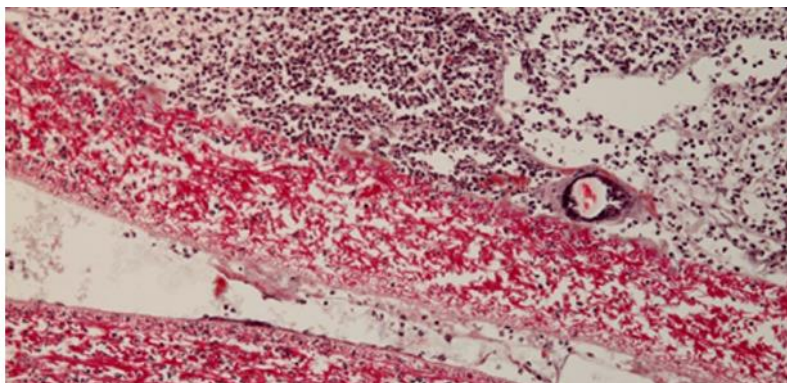


Figure 2: Microscopic image of the eggshell membrane with a highly polymorphic and irregular mesh structure resulting from pressure of edema fluid on the shell membrane fibers at the implantation site.^[5]

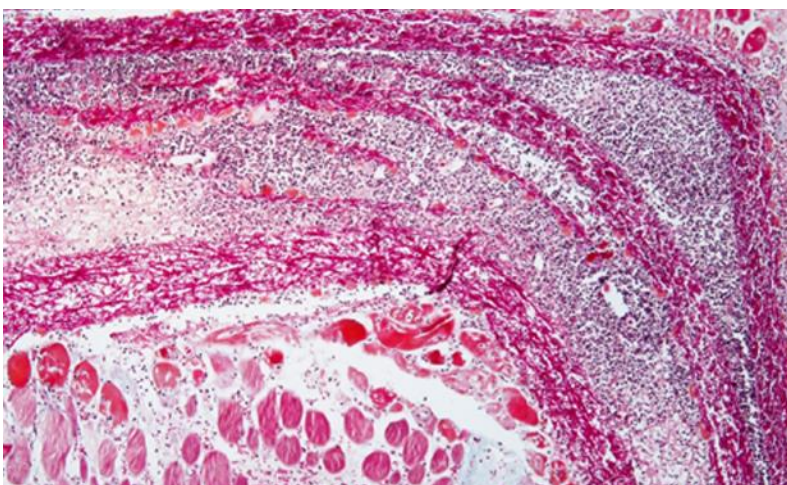


Figure 3: Microscopic image of the eggshell membrane implanted in muscle tissue, surrounded by moderate edema and abundant cellular infiltrate in the immediate vicinity of the membrane, gradually becoming less concentrated as the distance from the membrane increases.^[5]

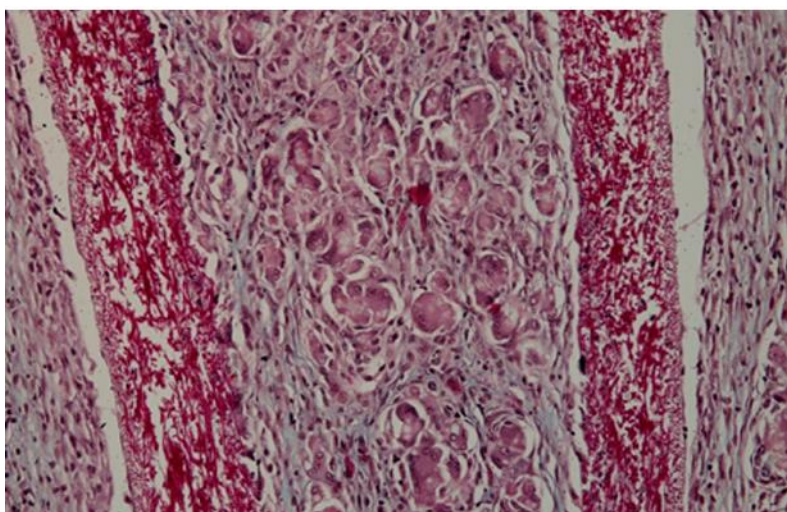


Figure 4: Microscopic image showing newly proliferating collagen fibers and multinucleated giant cells in the connective tissue surrounding the shell membrane, with variation in the number of cells observed between different cases and areas.^[5]

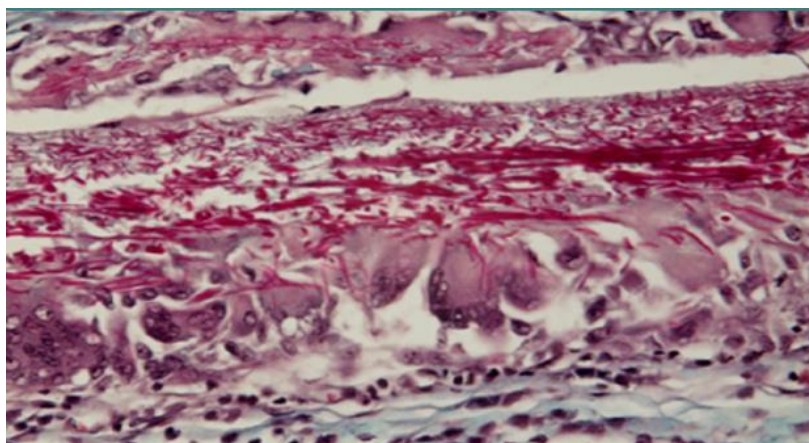


Figure 5: Microscopic image showing enlarged interfibrillar spaces at the level of the shell membrane due to a slow process of fibrinolysis, causing fragmentation of the membrane fibers.^[5]

Osteoclastic activity and remodeling are equally essential: after initial woven bone deposition, coordinated RANK/RANKL/OPG signaling controls osteoclast recruitment and function to replace immature bone with lamellar, load-bearing cortical and trabecular architecture. Material composition modulates osteoclastogenesis for example, persistent particulate debris or highly resorbable matrices that dissolve too rapidly can provoke exaggerated osteoclastic resorption or unstable remodeling. nCaSO₄ typically resorbs faster than hydroxyapatite, which can be advantageous for timely remodeling but requires balancing to avoid loss of structural support before sufficient lamellar bone is formed; controlling particle size and crystallinity (nano vs micro) adjusts resorption kinetics. PRF indirectly influences remodeling by promoting initial bone formation and vascularity, which supports osteocyte survival and establishment of mechanotransductive signaling that guides remodeling. At the histologic level, successful EnHA+nCaSO₄+PRF graft sites show early osteoid seams with embedded osteoblasts, subsequent osteocyte lacunae within maturing bone, and a progressive decrease in TRAP-positive multinucleated osteoclasts as lamellar bone consolidates. Monitoring these markers (ALP, osteocalcin, TRAP, histomorphometry) provides objective evidence of constructive remodeling and graft incorporation in experimental studies.^[5,8]

The specific role of plasma cells and adaptive immunity in bone graft contexts deserves careful attention because their presence can indicate chronic antigen exposure or low-grade graft incompatibility. Plasma cells, deriving from activated B lymphocytes, secrete specific immunoglobulins that can opsonize particulate debris and modulate macrophage phenotypes; a sustained plasma-cell infiltrate in graft biopsies correlates with prolonged inflammation and sometimes diminished bone formation in allogeneic or contaminated grafts.^[9,13] In contrast, autologous components such as PRF reduce the need for foreign protein carriers and therefore lower the risk of antigen-driven plasma cell responses; the leukocyte content of PRF also contributes antimicrobial peptides and immune modulators that help resolve bacterial contamination risk. Eggshell-derived materials, when properly processed and sterilized (removal of organic membranes and endotoxins), exhibit low immunogenicity, but surface impurities or organic residues can provoke lymphoplasmacytic infiltrates, hence rigorous material characterization is essential. Engineering strategies that promote M2 macrophage polarization and limit FBGC formation (for instance, nanostructuring surfaces and controlling ion release) appear to reduce plasma-cell recruitment and favor regenerative pathways. Therefore, combining EnHA, controlled-resorption nCaSO₄ and autologous PRF is biologically rational because it minimizes antigenic load while harnessing pro-regenerative immune signaling.^[14]

Finally, translating these mechanistic insights into robust clinical protocols requires standardization and rigorous outcome metrics. Key technical variables include EnHA particle size and porosity, nCaSO₄ crystallinity and degradation rate, the PRF centrifugation protocol (rpm/time), and the mixing ratio that yields workable “sticky bone” while preserving porosity for vascular ingrowth; small changes alter cellular responses (adhesion, proliferation, cytokine profiles) measurable in vitro and in vivo. Clinically, endpoints should combine volumetric CBCT analysis (bone volume and Hounsfield units), implant stability (ISQ or removal torque), and histomorphometric indices (BV/TV, osteoid percentage, TRAP counts) at standardized timepoints (e.g., 3, 6, 12 months).^[15] Safety monitoring must include signs of chronic lymphoplasmacytic inflammation, foreign-body reactions, and adverse remodeling; biopsies in a subset of patients or animal models are essential to correlate imaging with microscopic healing.^[16,17,18]

CONCLUSION

The combination of eggshell-derived nano-hydroxyapatite, nano calcium sulfate, and platelet-rich fibrin demonstrates promising short-term improvements in bone volume, radiographic density, and early mechanical stability in preclinical models and small clinical pilots. Mechanistically, this composite leverages osteoconductive mineral scaffolds (EnHA), a resorbable ionic stimulus and temporary structural support (nCaSO₄), and autologous growth-factor delivery with enhanced angiogenesis and cell recruitment (PRF). However, current evidence is limited by small sample sizes, heterogeneous materials and PRF protocols, and short follow-up periods, which preclude definitive conclusions about long-term remodeling and functional outcomes. Rigorous, standardized randomized trials and well-controlled preclinical studies with harmonized preparation methods, quantitative imaging, and histomorphometric endpoints are therefore required before routine clinical adoption.

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The authors declare that they have no conflicts of interest.

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