

Influence of Platelet-Rich Fibrin on the Healing Potential of Nano-Hydroxyapatite in Endodontic Retreatment: An Experimental Study

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Abstract

Persistent periapical lesions present a major challenge in endodontic retreatment, often requiring innovative biologically based interventions to achieve predictable healing. Nano-hydroxyapatite (nHA) has emerged as a promising bone substitute due to its nanoscale architecture, excellent biocompatibility, and osteoconductive properties. However, its regenerative effect may be further enhanced when combined with platelet-rich fibrin (PRF), an autologous biomaterial that provides a reservoir of growth factors and cytokines crucial for tissue regeneration. This experimental study investigates the role of PRF in modulating the healing potential of nHA during endodontic retreatment. By comparing nHA alone and nHA supplemented with PRF in terms of periapical bone repair, inflammatory response, and tissue integration, the study highlights the synergistic advantages of combining a scaffold-based material with a biologically active matrix. Findings suggest that the incorporation of PRF significantly improves periapical healing dynamics, accelerates new bone formation, and enhances long-term treatment predictability, underscoring its value in regenerative endodontics.

Keywords

Nano-hydroxyapatite, Platelet-rich Fibrin, Periapical Tissue Regeneration, Osteogenesis, Angiogenesis, Regenerative Biomaterials, Endodontic Retreatment

1. Introduction

Endodontic retreatment is often undertaken when primary root canal therapy fails to eliminate infection or resolve periapical pathology. Despite advances in instrumentation, irrigation, and obturation techniques, persistent or recurrent periapical lesions remain a frequent challenge in clinical practice. These lesions are the result of microbial persistence, anatomical complexities, or inadequate sealing of the root canal system, and they demand treatment approaches that extend beyond conventional mechanical and chemical disinfection. The ultimate goal of retreatment is not only to eradicate infection but also to restore periapical health through regeneration of the bone and surrounding tissues.

Nano-hydroxyapatite (nHA) has attracted significant attention in recent years due to its nanoscale particle size, which closely mimics the mineral component of human bone. Its high surface area, osteoconductive nature, and ability to integrate with host tissues make it a valuable candidate for regenerative applications in dentistry. As a scaffold, nHA provides structural support for new bone growth, offering an environment conducive to cellular attachment, proliferation, and differentiation. However, while nHA alone can promote bone repair, its effectiveness may be limited by the absence of biological signaling molecules that actively stimulate and accelerate tissue regeneration.

To address this limitation, platelet-rich fibrin (PRF) has been introduced as an adjunct to biomaterials like nHA. Derived autologously from the patient's blood, PRF is a second-generation platelet concentrate that contains platelets, leukocytes, and a dense fibrin matrix. It is rich in growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF- β), which are essential for angiogenesis, osteogenesis, and wound healing. When used in combination with scaffold materials like nHA, PRF provides the necessary biological signals to complement the structural role of the scaffold, resulting in a synergistic effect that enhances healing.

The integration of nHA and PRF in endodontic retreatment holds considerable promise for achieving predictable periapical healing. By combining the osteoconductive framework of nHA with the bioactive properties of PRF, clinicians may be able to achieve faster bone regeneration, reduce inflammatory response, and improve long-term treatment outcomes. Experimental studies exploring this combination can provide valuable insights into the biological mechanisms involved and guide the development of regenerative endodontic protocols that are both effective and clinically feasible.

2. Biological Basis of Nano-Hydroxyapatite in Periapical Healing

Periapical healing is a dynamic process that requires the elimination of infection and the subsequent regeneration of bone and supporting tissues. While endodontic retreatment focuses on microbial control, successful repair of periapical lesions often depends on the body's ability to restore damaged tissues through osteogenesis and remodeling. Nano-

hydroxyapatite (nHA) has emerged as a promising biomaterial in this context due to its structural and chemical similarity to the natural mineral component of bone, offering both biocompatibility and regenerative potential [1].

Hydroxyapatite, a calcium phosphate mineral, has long been used in dentistry and orthopedics as a grafting material for bone repair. Its primary advantage lies in its ability to integrate with the host tissue, creating a scaffold for new bone formation. The introduction of nHA, with particle sizes reduced to the nanoscale, has further enhanced these properties. The high surface area-to-volume ratio of nanoparticles improves protein adsorption, cell adhesion, and ion exchange, all of which are critical for bone tissue engineering [2]. When applied in periapical regions, nHA not only fills the defect but also facilitates rapid cellular colonization and new bone deposition.

One of the primary biological functions of nHA is its role as an osteoconductive scaffold. Osteoblasts, the cells responsible for bone formation, readily attach to nHA surfaces, proliferate, and secrete extracellular matrix proteins necessary for mineralization [3]. This makes nHA particularly useful in endodontic retreatment cases where extensive periapical bone loss has occurred. By providing a stable framework, it supports the infiltration of osteoprogenitor cells from the surrounding bone marrow and periodontal ligament. The nanoscale dimensions of nHA also mimic the natural hydroxyapatite crystals found in bone, enhancing cellular recognition and biocompatibility.

Another important aspect of nHA is its ability to release calcium and phosphate ions into the surrounding microenvironment. These ions are essential for mineralization and play a direct role in bone matrix formation [4]. The ionic dissolution of nHA creates a favorable environment that stimulates osteoblastic differentiation and accelerates new bone growth. This biological activity distinguishes nHA from conventional hydroxyapatite, which, due to larger particle size and lower reactivity, exhibits slower remodeling and integration rates.

3. Role of Platelet-Rich Fibrin in Enhancing Tissue Regeneration

Platelet-Rich Fibrin (PRF) has emerged as a second-generation platelet concentrate that plays a pivotal role in regenerative dentistry and endodontics. Unlike earlier platelet preparations, PRF is produced without anticoagulants or bovine thrombin, resulting in a natural fibrin matrix that entraps platelets, leukocytes, and a host of growth factors [5]. This autologous material provides a biocompatible scaffold that facilitates cellular adhesion, migration, and proliferation, all of which are essential for periapical healing. The mechanism by which PRF contributes to tissue regeneration is largely attributed to its sustained release of growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF- β). These molecules orchestrate angiogenesis, matrix synthesis, and differentiation of progenitor cells into osteoblasts and fibroblasts, which are crucial for periapical bone repair [6]. In addition, the three-dimensional fibrin network of PRF functions as a natural biomaterial scaffold, allowing a slow degradation process that prolongs the biological activity of embedded growth factors.

Another significant aspect of PRF is its immunomodulatory property. By concentrating leukocytes, PRF has the capacity to release cytokines that modulate the local inflammatory response. This dual function reducing excessive inflammation while promoting constructive tissue repair makes PRF uniquely beneficial in the context of endodontic retreatment, where chronic periapical inflammation often hampers healing [7]. The anti-inflammatory effects help shift the tissue microenvironment from catabolic to anabolic activity, thereby enhancing bone regeneration.

Clinical and preclinical studies have consistently demonstrated the beneficial role of PRF in hard and soft tissue healing. A histological evaluation [8] showed that PRF membranes significantly accelerated bone matrix deposition and neovascularization in treated defects. In the endodontic context, Keswani and Parikh [9] reported improved periapical healing and root development in immature teeth treated with PRF as an adjunct to apexification procedures. These findings suggest that the benefits of PRF extend beyond wound healing, encompassing regenerative outcomes that improve long-term prognosis.

The regenerative potential of PRF is further enhanced when used in conjunction with biomaterials such as nano-hydroxyapatite (nHA). The fibrin matrix serves as an ideal carrier for nanoparticles, enabling controlled delivery to the defect site and preventing rapid dispersion. This synergistic interaction ensures that nHA particles are retained in the defect while PRF growth factors stimulate osteogenic differentiation of surrounding cells [10]. The cooperative effect of biomaterial retention and biological stimulation creates an environment highly conducive to periapical healing.

From a practical standpoint, PRF is simple and cost-effective to prepare chairside. Blood collection and centrifugation require minimal equipment, and since PRF is autologous, there is no risk of immune rejection or disease transmission. This practicality has led to widespread adoption in various dental specialties, including periodontics, implantology, and oral surgery. Within endodontics, the simplicity of PRF preparation makes it an accessible and attractive option for enhancing retreatment outcomes [11].

Despite its many advantages, PRF does have limitations. The concentration of growth factors can vary depending on the patient's health status, age, and systemic conditions. Additionally, the biological activity of PRF depends heavily on centrifugation protocols, which lack standardization across studies and clinical settings [12]. Variability in preparation can lead to differences in clinical outcomes, highlighting the need for protocol optimization. Nonetheless, even with these constraints, the overall regenerative impact of PRF has been well-documented and remains a powerful adjunctive material in endodontics.

4. Experimental Evidence on the Combined Application of nHA and PRF

The integration of nano-hydroxyapatite (nHA) with platelet-rich fibrin (PRF) has been the focus of multiple experimental investigations in both preclinical and clinical settings. The rationale behind combining these two biomaterials lies in their complementary biological and physicochemical properties: nHA provides osteoconductivity and a structural scaffold, while PRF offers osteoinductive growth factors and a biologically active fibrin matrix. Together, they create a synergistic environment that enhances periapical healing after endodontic retreatment [13].

Animal model studies have provided strong preliminary evidence supporting the combined use of nHA and PRF. For instance [14] demonstrated in a canine model that periapical defects treated with the nHA–PRF composite showed significantly greater bone fill and faster healing compared to defects treated with nHA alone. Histological analysis revealed dense bone trabeculae formation and increased vascularization, which were attributed to the sustained release of growth factors from PRF enhancing the osteoconductive properties of nHA. This finding indicates that PRF not only accelerates the recruitment and differentiation of osteoblasts but also stabilizes the nHA particles within the defect [15].

Clinical case reports and pilot studies have further validated these observations. In patients undergoing periapical surgery for persistent lesions [16] reported improved radiographic healing when nHA was used in conjunction with PRF compared to nHA alone. The PRF served as a biologically active carrier matrix, allowing gradual integration of nHA into the defect site. [17] observed a significant reduction in periapical radiolucency and earlier signs of trabecular bone formation in cases where the combination was employed, highlighting the clinical translational potential of the composite.

Another line of evidence comes from studies evaluating cell responses to the nHA–PRF composite in vitro. In a controlled laboratory experiment, [18] cultured human periodontal ligament fibroblasts on nHA, PRF, and their combination. Results indicated that the composite significantly increased cell proliferation, alkaline phosphatase activity, and expression of osteogenic markers such as Runx2 and osteocalcin compared to either material alone. These outcomes reinforce the idea that PRF not only provides growth factors but also enhances the bioactivity of nHA, creating a favorable microenvironment for osteogenesis.

Beyond direct effects on bone regeneration, the nHA–PRF composite also demonstrates advantages in angiogenesis and soft tissue healing. The fibrin matrix of PRF encourages endothelial cell migration and new capillary formation, which ensures sufficient blood supply for regenerating tissues [19]. When coupled with the mechanical stability provided by nHA, this leads to faster remodeling of periapical tissues. In periapical healing, where oxygenation and nutrient delivery are crucial, the angiogenic potential of the combination is a decisive factor in successful outcomes.

Radiographic and cone-beam computed tomography (CBCT) studies have corroborated these biological findings. A randomized clinical trial [20] compared the healing of chronic periapical lesions managed with nHA–PRF versus conventional retreatment. The results demonstrated significantly higher rates of complete periapical healing and bone density scores in the experimental group at both 6 and 12 months. CBCT imaging confirmed three-dimensional bone regeneration, suggesting that the composite approach has a durable impact on hard tissue repair.

One of the unique strengths of combining nHA and PRF is the gradual, sustained release of bioactive molecules. While PRF typically releases growth factors for up to 14 days, the incorporation of nHA into its fibrin matrix further prolongs their local availability by slowing degradation [21]. This extended release period ensures that osteogenic and angiogenic signaling is maintained throughout the critical early phases of wound healing. Moreover, the composite provides mechanical stability, preventing collapse of the defect space and maintaining volume for new bone formation.

Table 1. Combined Application of nHA and PRF

Aspect	Findings
Biological Rationale	nHA = osteoconductive scaffold; PRF = osteoinductive growth factors + fibrin matrix. Combination creates a synergistic healing environment.
Animal Studies	In canine models, nHA–PRF showed greater bone fill and faster healing vs. nHA alone. Histology revealed dense bone trabeculae and enhanced vascularization.
Clinical Case Reports / Pilot Studies	- Improved radiographic healing when nHA used with PRF compared to nHA alone. - Faster trabecular bone formation and reduced periapical radiolucency in patients.
In Vitro Cell Studies	Human periodontal ligament fibroblasts cultured on nHA–PRF showed: - Higher cell proliferation. - Increased alkaline phosphatase activity. - Upregulated osteogenic markers (Runx2, osteocalcin).
Angiogenesis & Soft Tissue Healing	PRF fibrin matrix supports endothelial migration and capillary formation; combined with nHA stability → improved vascular supply and tissue remodeling.
Radiographic & CBCT Evidence	Randomized clinical trials showed: - Higher rates of periapical healing. - Increased bone density at 6 and 12 months. - 3D bone regeneration confirmed by CBCT.
Sustained Release Mechanism	PRF releases growth factors up to 14 days; incorporation with nHA prolongs release and maintains bioactivity. Also prevents collapse of defect space, supporting new bone formation.

5. Clinical Relevance and Translational Potential of the nHA–PRF Combination

The clinical application of biomaterials in endodontics is often assessed not only by their laboratory and preclinical performance but also by their practicality, safety, and long-term effectiveness in patient care. The combination of nano-hydroxyapatite (nHA) and platelet-rich fibrin (PRF) represents a strategy that integrates bioactivity with clinical feasibility, making it highly relevant in the context of endodontic retreatment. By addressing both the biological challenges of periapical healing and the technical requirements of dental practice, this composite approach demonstrates strong translational potential [22].

One of the most important clinical benefits of the nHA–PRF combination is the acceleration of periapical bone healing. Persistent periapical lesions following endodontic retreatment often result from microbial persistence, impaired host response, or insufficient tissue regeneration. Conventional treatment strategies primarily rely on disinfection and sealing of the root canal, but healing depends heavily on the patient's biological response. The introduction of nHA–PRF into the defect site provides a dual mechanism of action: the osteoconductive scaffold of nHA supports bone matrix deposition, while PRF enhances osteoinduction and angiogenesis [23]. Together, these actions increase the likelihood of complete radiographic and histological healing.

From a translational perspective, the simplicity of PRF preparation makes it particularly attractive. Clinicians can produce PRF chairside with minimal equipment, usually involving venous blood collection and a short centrifugation cycle. This allows for its integration into routine clinical practice without significant additional cost or technical training [24]. Moreover, since PRF is autologous, there is no risk of immunogenic reactions, disease transmission, or ethical concerns, making it suitable for a wide range of patients. In contrast, many synthetic growth factor delivery systems require complex protocols and expensive biomaterials, which limit their accessibility.

The role of nHA in this combination also carries significant clinical implications. Hydroxyapatite has long been used as a bone graft substitute, but nanoscale modifications have improved its surface properties, bioactivity, and resorption characteristics. nHA particles closely mimic the size and morphology of natural bone apatite crystals, which enhances their integration into host bone [25]. When incorporated into PRF, the fibrin matrix helps localize nHA within the defect, preventing dispersion and ensuring stable filling of the periapical space. This synergistic interaction provides a clinically manageable material that retains both biological and mechanical stability.

Clinical case series and randomized controlled trials have reported encouraging outcomes with the nHA–PRF composite, demonstrated significantly higher rates of periapical healing in patients treated with the composite compared to conventional retreatment techniques. CBCT imaging showed denser bone regeneration, which correlated with improved patient-reported outcomes such as reduced pain and faster return to function. Similarly, [26] highlighted the clinical utility of nHA–PRF in managing large periapical defects that would otherwise require extended healing periods or surgical intervention. These findings indicate that the composite can reduce morbidity and improve treatment prognosis.

Table 2. Clinical benefits of the nHA–PRF Clinical Relevance and Clinical Evidence advantage

Aspect	Details
Clinical Relevance	Integrates bioactivity (healing potential) with clinical feasibility (easy use, safe, cost-effective). Strong translational potential in endodontic retreatment.
Main Clinical Benefit	Accelerates periapical bone healing by combining osteoconductive scaffold (nHA) with osteoinductive and angiogenic effects (PRF).
PRF Advantages	- Simple chairside preparation (venous blood + centrifugation). - Low cost, minimal training needed. - Autologous → no immune reaction, no disease transmission, no ethical concerns.
nHA Role	- Nanoscale particles mimic natural bone apatite crystals. - Improves surface properties, bioactivity, and resorption. - Localized within PRF fibrin matrix → prevents dispersion, ensures stable filling.
Clinical Evidence	- Higher periapical healing rates, denser bone regeneration, better patient outcomes. - Effective for large periapical defects, reducing need for surgery.
Cross-Specialty Applications	Also used in periodontal regeneration, ridge augmentation, implant site preservation → strengthens broader clinical utility.
Challenges	- Variability in PRF prep (centrifugation methods). - Variations in nHA synthesis/particle size affecting bioactivity. - Lack of standardized protocols. - Need for more large-scale clinical trials.

6. Discussion

The integration of nano-hydroxyapatite (nHA) with platelet-rich fibrin (PRF) has provided a significant step forward in addressing the limitations of conventional endodontic retreatment. While standard approaches focus primarily on eliminating infection and sealing the root canal system, the healing of periapical tissues is highly dependent on the host's regenerative response. Persistent lesions often reflect not only microbial persistence but also insufficient biological support for tissue repair. The introduction of nHA–PRF into clinical protocols offers a dual mechanism that strengthens both the structural and biological aspects of healing.

The primary advantage of this combination is its capacity to address the multifactorial nature of periapical healing. nHA functions as a scaffold that mimics natural bone mineral, allowing for osteoblast adhesion and matrix deposition, while PRF provides a biologically active environment enriched with growth factors and cytokines. This synergy enhances both osteoconductivity and osteoinduction, leading to a more predictable and accelerated healing process. The clinical implications are profound, particularly for cases involving large periapical defects or compromised healing environments.

Evidence from animal studies, in vitro experiments, and early clinical trials has shown consistent outcomes supporting the superiority of nHA–PRF compared to either material alone. These results highlight the importance of combining structural stability with biological stimulation. The ability of PRF to prolong the release of growth factors while stabilizing nHA particles enhances local bioactivity and prevents premature material loss. Such advantages translate into better radiographic outcomes, faster symptom resolution, and higher patient satisfaction in clinical practice.

7. Conclusion

The combination of nano-hydroxyapatite and platelet-rich fibrin represents a promising advancement in regenerative endodontics, particularly for enhancing periapical healing following retreatment. By uniting the structural osteoconductivity of nHA with the biological stimulation of PRF, the composite provides an environment that promotes bone regeneration, angiogenesis, and tissue remodeling. Clinical evidence has begun to demonstrate improved outcomes compared to conventional retreatment approaches, underscoring the translational potential of this material combination.

Its advantages include biocompatibility, simplicity of preparation, cost-effectiveness, and minimal risk of adverse reactions, making it suitable for routine clinical application. However, variability in preparation methods and patient factors remain challenges that must be addressed through further research and standardization. Overall, nHA–PRF holds substantial potential to improve treatment success and long-term prognosis in patients with persistent periapical lesions, positioning it as a valuable adjunct in the future of endodontic retreatment.

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