

Research Article

Use of Circularly Polarized Light on the Evaluation of Injectable Platelet Rich Fibrin in the Early Formation of Woven and Lamellar Bone: A Specific Method in the Study of Osseous Tissue

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Abstract

Purpose: In bone grafting, the goal is the formation of 100% vital bone. The ideal graft material is osteogenic, osteoconductive and osteoinductive and can be evaluated by histology and histomorphometric methods to calculate percentages of vital bone formation, residual graft material and connective tissue. Bone graft substitute materials currently used include allogeneic, xenogeneic and alloplastic materials. The goal of this study is a histological evaluation of the early effects of the liquid (injectable) form of Platelet Rich Fibrin (i-PRF) on the formation of woven and lamellar bone using Circularly Polarized Light (CPL).

Patients and Methods: This is a retrospective cohort study of forty-two patients from 2021 to 2022 who completed bone grafting after a non-restorable endodontically treated molar tooth was extracted in preparation for future implant surgery. Each bone graft was augmented with i-PRF. To process i-PRF, Relative Centrifugation Force (RCF) was reduced from 2,700 RPM to 700 RPM (60 g). Centrifugation time was reduced from 12 minutes to 3 minutes. After an average bone graft healing time of 8 to 12 weeks, bone core samples were obtained at the time of implant surgery for histological and histomorphometric analysis. Bone cores were stained with Stevenel's blue and van Gieson's picrofuchsin for histologic analysis by means of brightfield and circular polarized light microscopic evaluation to evaluate the early effects of the liquid form of PRF on formation of woven and lamellar bone using allogeneic bone.

Results: Forty-two bone cores were harvested at the time of implant surgery for histological and histomorphometric analysis. The liquid form of PRF resulted in greater percentages of vital bone formation (average 87.4 %) compared to the existing published literature. CPL analysis of forty-two bone core specimens showed the positive effects of i-PRF on de novo bone formation.

Conclusion: The results of this study using circularly polarized light demonstrate that the liquid form of PRF (i-PRF) resulted in greater percentages of early, new vital bone formation

compared to the published literature. Circularly polarized light should be considered as an adjunctive method to determine bone maturity and structure.

Keywords: Circularly Polarized Light; Liquid (Injectable) Platelet Rich Fibrin (i-PRF); Early Bone Formation; Allogeneic Bone; Woven Bone; Lamellar Bone

Introduction

Bone graft augmentation is a common surgical procedure to correct the deficient or atrophic jaw in preparation for dental implant surgery [1,2]. De novo bone formation initially occurs by formation of woven (immature) bone that acts as a scaffold for deposition of lamellar (mature) bone [3-5]. The formation of mineralized bone results in specific patterns of self-assembly, with spatial and temporal characteristics. Two distinct osteoblast cell lines are responsible for osteogenesis. Surrounding mesenchymal osteoblast in a random circumferential pattern are collagen fibrils. In a linear array pattern, surface osteoblasts are stacked on to the surface of the mesenchymal osteoblasts to synthesize parallel fibered lamellar bone.

During osteogenesis, four key events of woven bone formation have been identified [5]. The initial stage of woven bone formation is marked by the formation of pre-osteoblastic cells from undifferentiated mesenchymal cells, followed by the formation of randomly oriented matrix fibers that surround mesenchymal cells in a 360-degree circumference. The third stage is characterized by surface osteoblast synthesizing parallel fibered lamellar bone on the woven bone scaffold. The final stage of osteogenesis is characterized by diminution of the woven matrix bone.

In a previous published research paper using conventional histologic and histomorphometric analysis, Lee, et al., showed that the liquid form of Platelet Rich Fibrin (i-PRF) resulted in the early formation of new vital bone in mandibular molar extraction sites using allogeneic bone in preparation for implant surgery [6]. After an average bone graft healing time of 8 to 12 weeks, bone core samples were obtained at the time of implant surgery for histological and histomorphometric analysis. Forty-two bone cores were harvested at the time of implant surgery for histological and histomorphometric analysis. The liquid form of PRF resulted in greater percentages of vital bone formation (average 87.4%) compared to the existing published literature.

Analysis of bone formation requires precise scientific information. A method to evaluate new bone formation is histologic examination. However, it can be challenging with standard histological techniques to view woven (immature) from lamellar (mature) bone [7-9]. Different laboratory methods have been developed to study the microarchitecture of osseous tissue, specifically circularly polarized light microscopy [9-11].

Circularly polarized light microscopy forms an image due to refraction of light at multiple indices, known as birefringence. In osseous tissue, birefringence is due to collagen orientation or increased density [11]. Evaluation of the pattern of birefringence can overcome these difficulties to differentiate mature and immature bone. Further, it can be difficult to distinguish vital bone from bone graft substitutes. To overcome these challenges, Circularly Polarized Microscopy (CPL) is a specific laboratory method to determine bone maturity and structure. The goal of this study was a histological evaluation using circularly polarized light microscopy on the early effects of i-PRF on de novo bone formation using allogeneic bone.

Circularly Polarized Light (CPL) Microscopy

Bone provides many functions to the human body and is dependent on both the quality and quantity of osseous tissue. It is also a function of bone geometry, its microarchitecture and its mineral and collagen fiber cross-linking orientation [7]. This contributes to the biomechanical characteristics of bone. Identification of bone tissue is the primary function of histologic evaluation [8]. Using polarized light microscopy, woven and lamellar bone can be differentiated from each other and if often due to the birefringence of collagen [11].

Collagen fibrils are birefringent. A technique for measuring birefringence of lamellar bone is with the use of the circularly polarized light microscopy due to the birefringence of collagen [9-11]. Polarized light microscopes are overly sensitive and can improve the quality of the image of birefringent images. Polarized light microscopes have two linear polarizers that are perpendicular to each other that do not permit light to pass through them with the presence of a tissue specimen. When an unstained anisotropic tissue specimen such as bone is placed between the two polarizers, the state of polarization is altered and light can pass through the second polarizer. Birefringence can be determined by measuring images in the grayscale and is used

to determine collagen fiber orientation [12]. Type I collagen fibers are present in the extracellular matrix of bone produced by osteoblasts during collagen synthesis and provide bone flexibility and strength.

Generation of CPL is accomplished with a quarter-wave retarder and two polarizing filters. Each bone specimen is placed between a circular polarizing filter. The quarter-wave retarder is placed below the analyzer. Collagen fiber orientation was examined using different resolution levels by rotating the analyzer from -5 to +5 degrees per image that was analyzed. Orientation of collagen fibers were evaluated using a NIKON ECLIPSE 50i microscope (Nikon Corp., Japan) and a SPOT INSIGHT 2 mega sample digital camera (Diagnostic Instruments, Inc., USA) [13].

Material and Methods

Study Design and Sample

To address the goals of this study, the authors designed a cohort study to evaluate the effects of the liquid form of PRF on the formation of woven and lamellar bone using circularly polarized light. During this study, forty-two patients (10 male and 32 female) were enrolled in this clinical case series and surgery completed in a private practice (CYSL). Their age range was 42 to 78 years. The mean age was 63 years old. Bone cores were obtained 8 to 12 weeks, post-surgery. This study was in accordance with the ethical standards of the institutional and national research committee and with the Helsinki Declaration of 1975 that was revised in 2000 [14]. Informed consent was obtained from all patients in this study and deidentified.

Preparation of i-PRF and Bone Graft Material

Venous blood was obtained from the antecubital fossa of the upper extremity from each patient enrolled in the study. Blood was collected in two 10 ml sterile glass tubes without the addition of any anticoagulant and immediately placed in the centrifuge for processing of liquid L-PRF. Centrifugation parameters were set at 700 rpm for 3 minutes that resulted in approximately 1 ml of the liquid form of L-PRF at the most superior layer. As i-PRF is produced as a liquid, it was aspirated from the glass tubes using a 25-gauge sterile needle attached to a 3 ml plastic syringe. The liquid form of L-PRF is to be sprayed on to the graft material before the graft material is mixed and placed in the bone graft recipient site.

Surgical Operative Technique

All patients rinsed with chlorhexidine gluconate 0.12% prior to the surgical procedure for 45 seconds. Under local anesthesia, surgical extraction of endodontically treated first and second mandibular molars was completed by only one oral and maxillofacial surgeon (CYSL). With completion of the extraction of the molar tooth and preparation of the bone graft recipient site, 0.5 cc to 1.0 cc of mineralized cortical freeze-dried allogeneic bone (LifeNet Health, Virginia Beach, VA) was loosely packed into the molar recipient site up to the alveolar crest after the i-PRF was mixed with the graft material. The graft material was covered with a titanium-reinforced high-density Polytetrafluoroethylene (dPTFE) membrane (Cytoplast Ti-250 Titanium-Reinforced Dense Membrane, Osteogenics Biomedical, Lubbock, TX).

Histologic Processing

Bone core specimens were harvested and placed in 10% neutral buffered formalin immediately upon implant placement. Upon receipt at the Hard Tissue Research Laboratory at the University of Minnesota, bone specimens were sectioned vertically in an anterior and posterior orientation according to protocol specifications. Sectioned specimens were dehydrated with a graded series of alcohols for 9 days. After dehydration, the specimens were infiltrated with a light-curing embedding resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). After 20 days of infiltration with constant shaking at normal atmospheric pressure, the specimens were embedded and polymerized by 450-nm light with the temperature of the specimens never exceeding 40°C. Specimens were prepared by the cutting/grinding method of Rohrer and Schubert [14]. The specimens were cut to a thickness of 150 mm on an EXAKT cutting/ grinding system (EXAKT Technologies, Oklahoma City, OK). Specimens were polished to a thickness of 45 to 65 mm using a series of polishing sandpaper discs from 800 to 2400 grit using an EXAKT micro grinding system followed by a final polish with 0.3-mm alumina polishing paste. Slides were stained with Stevenel's blue and van Gieson's picrofuchsin and cover-slipped for histologic analysis by means of brightfield and circular polarized light microscopic evaluation. Microphotographs were obtained, scanned, digitized and analyzed using a NIKON ECLIPSE 50i microscope (Nikon Corp., Japan) and a SPOT INSIGHT 2 mega sample digital camera (Diagnostic Instruments, Inc., USA). Histomorphometric measurements were completed using a combination of programs SPOT INSIGHT 2 mega sample digital camera, (Adobe

Photoshop, Adobe Systems, USA) and a public domain image program (NIH Image, National Institutes of Health, USA).

Results

Histologic Analysis

The primary variable evaluated is the formation of woven bone and lamellar bone observed using circularly polarized light. Representative images of de novo bone formation using circular polarized light are illustrated in Fig. 1-3. Woven bone, lamellar bone and residue allogeneic bone were observed using CPL microscopy. Woven bone was observed by identification of a random collagen fiber pattern with weak birefringence. Further, bridging of woven and lamellar bone could be seen within the mineralized bone matrix (Fig. 3,4). Osteoid tissue was also observed on the surface of newly formed bone (Fig. 5,6). Using circular polarized light and positioned in the plane of the section, transverse collagen fibers appeared as yellow-orange. Perpendicular to the plane of the section, longitudinal collagen fibers appeared as shades of white-gray.

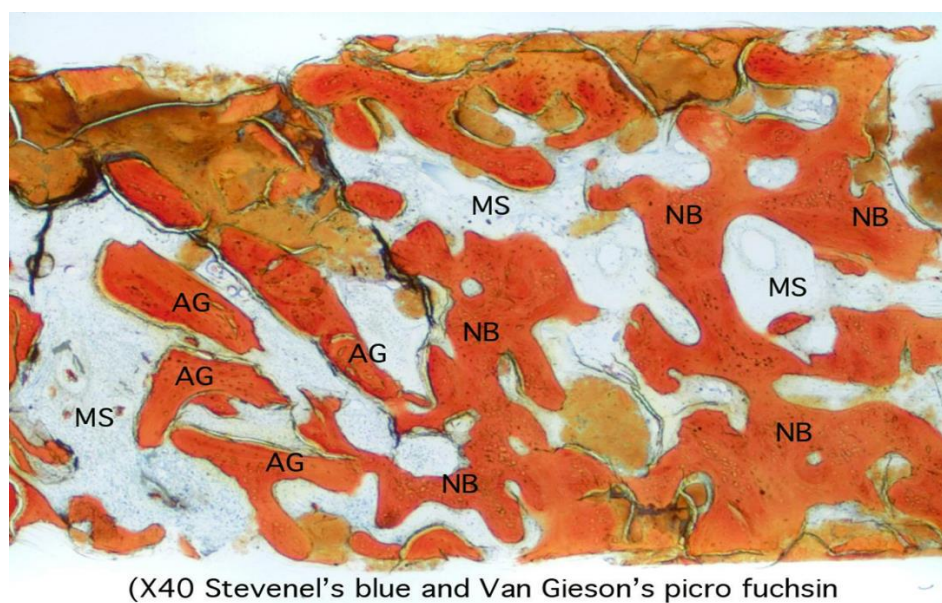


Figure 1: Medium power view at 8-weeks post-surgery of bone core showing newly formed woven bone designated as New Bone (NB). Particles of Allograft Bone (AG) and Marrow Spaces (MS) are observed. (x40, Stevenel's blue and Van Gieson's picro fuchsin).

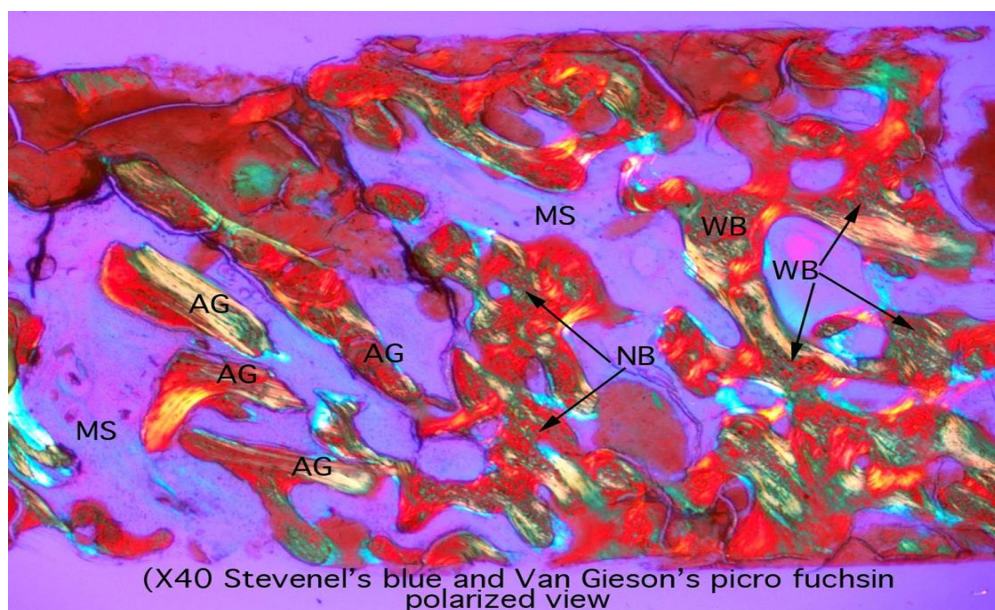


Figure 2: Circularly polarized light image at 8-weeks post-surgery differentiating mature New Bone (NB), immature Woven Bone (WB). Allograft particles (AG) and numerous Marrow Spaces (MS) are present. (x40, Stevenel's blue and Van Gieson's picro fuchsin).

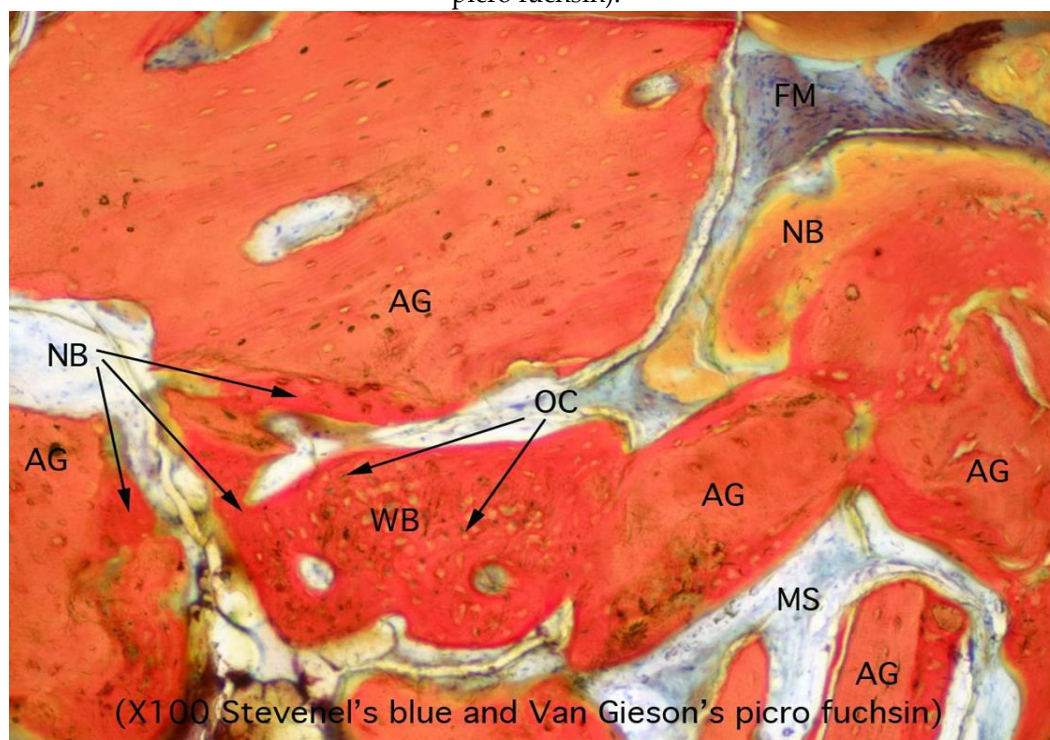


Figure 3: High power image of bone core at 8-weeks post-surgery showing immature Vital Bone (NB), Woven Bone (WB), Fibrous Marrow (FM) and Marrow Space (MS). Bridging between bone matrix and graft material are observed. Allograft bone particles embedded in bone, as well as attached to bone can be seen. (x100, Stevenel's blue and Van Gieson's picro fuchsin).

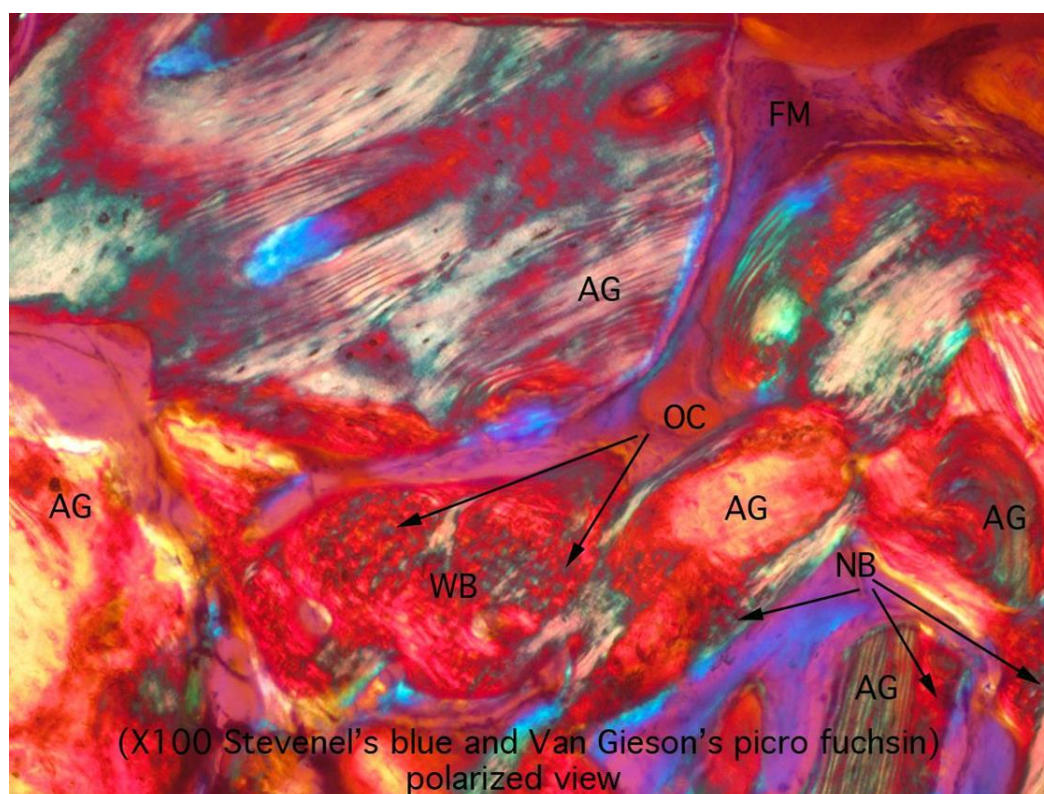


Figure 4: Circularly polarized light image at 8-weeks post-surgery showing the pattern of immature Woven Bone (WB), New Bone (NB), Osteocytes (OC) and Marrow Space (MS). The color observed in the polarized view is dependent on the orientation of the collagen fibers in the bone emphasizing a pattern of the immature bone that has not developed a lamellar mature pattern. (x100, Stevenel's blue and Van Gieson's picro fuchsin).

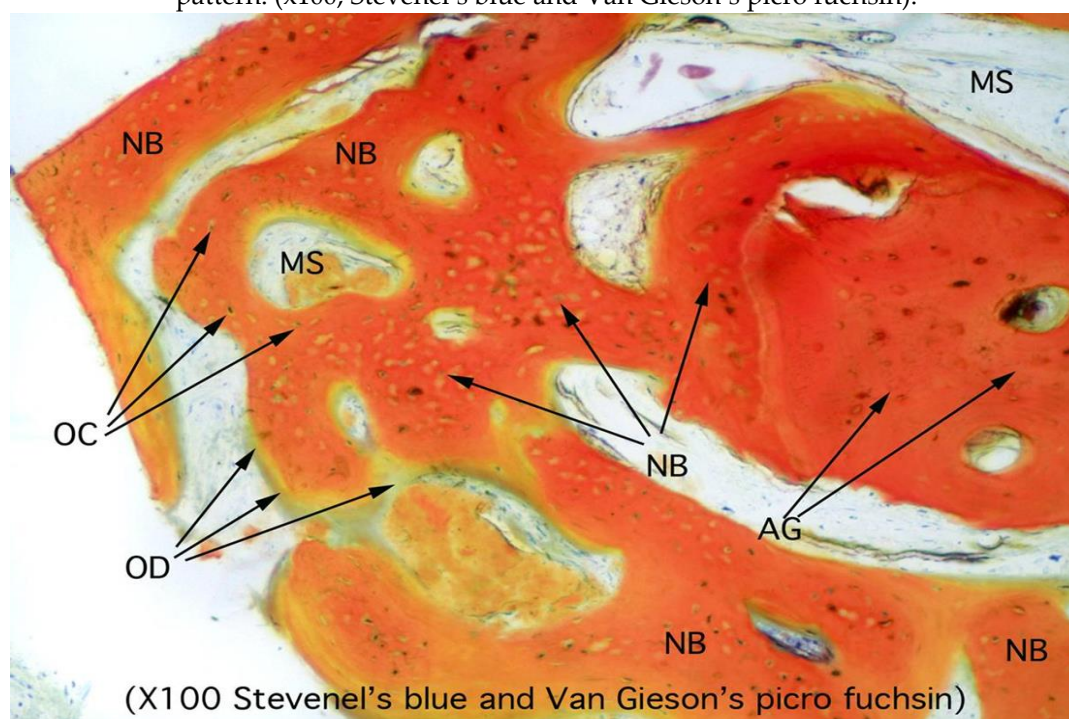


Figure 5: High power image at 12-weeks post-surgery revealing vital New Bone (NB) formation with Osteoid (OD), Osteocytes (OC) and Marrow Space (MS) present. Osteocytes (OC) in their lacunae are numerous and irregularly arranged in immature bone (NB). Also observed are mineralized Allograft (AG) outlined by arrows. (x100, Stevenel's blue and Van Gieson's picro fuchsin).

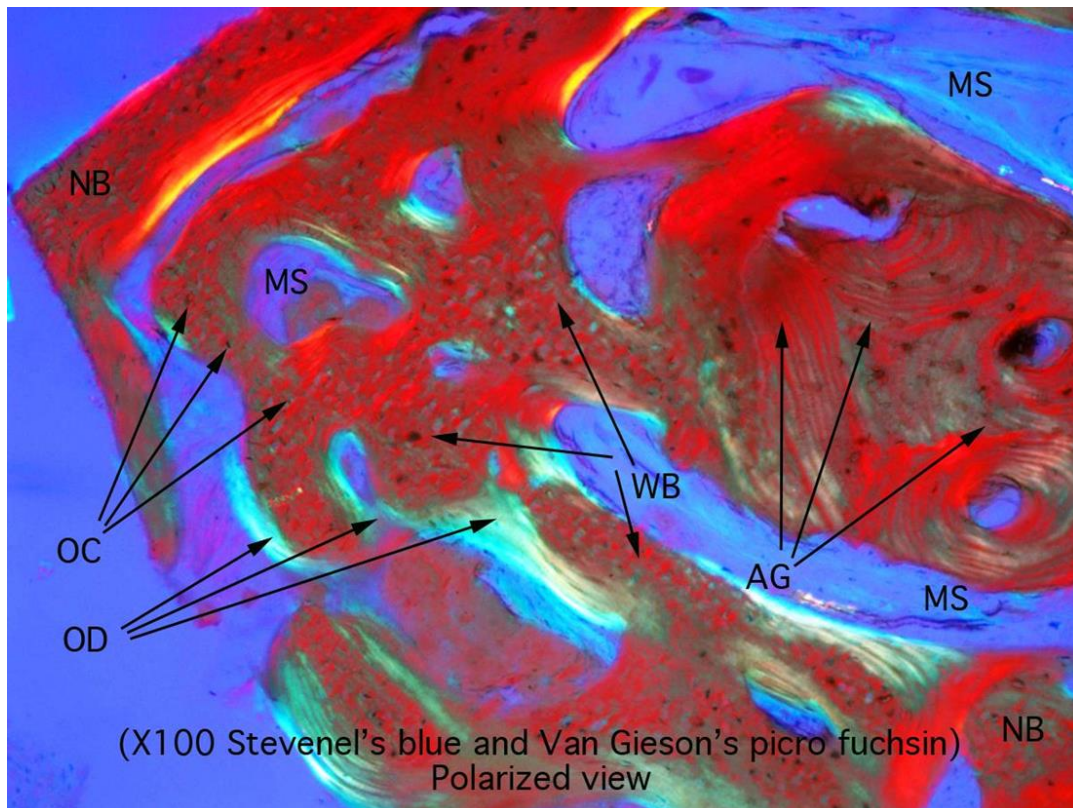


Figure 6: Circularly polarized light view at 12-weeks post-surgery showing the pattern of immature, “Woven” Bone (WB), Osteoid (OD), Osteocytes (OC) and marrow space (MS). Also observed is New Bone formation (NB) incorporated with Allograft particles (AG). (x100, Stevenel’s blue and Van Gieson’s picro fuchsin).

Discussion

To the best of the author’s knowledge, this is the first clinical study that specifically evaluated the histological effect of the liquid form of platelet rich fibrin on de novo bone formation using circularly polarized light microscopy. Platelets play a primary role in osteogenesis as they facilitate the early inflammatory phase and development of first woven, then lamellar bone [15]. Platelets contain lysosomes, dense granules and alpha granules [3,4,16]. With formation of the fibrin clot, the alpha granules release important bioactive molecules such as Platelet-Derived Growth Factors A and B (PDGF-AB), Transforming Growth Factor beta (TGF-B), Insulin-Like Growth Factor-1 (IGF-1), Epidermal Growth Factor (EGF) and Vascular Endothelial Growth Factor (VEGF) [17,18]. These bioactive molecules are all involved in neovascularization and differentiation of mesenchymal cells.

Although the published scientific literature supports the positive histologic effects of autologous platelet concentrates (PRP, PRF) on woven and lamellar bone formation, the exact mechanism remains unclear [19,20]. Using polarized light microscopy (Fig. 2-6), lamellar bone can be clearly differentiated from woven bone due to the collagen maturation pattern and enlarged elliptical shaped lacunae with their osteocytes present [8]. Mature bone appears as thick, parallel birefringent collagen fibers. In contrast, immature bone presents as weakly birefringent [21].

In our study using polarized light microscopy, we determined that i-PRF facilitates early woven and lamellar bone formation. Polarizing light revealed the collagen fiber orientation that differentiates the characteristic formation of lamellar and woven bone. PRF was also shown to positively affect osteoblastic differentiation leading to woven bone initially, followed by lamellar bone formation superimposed on woven bone.

Conclusion

To the best of our knowledge, this is the first clinical study that demonstrates the enhanced and positive effects of the liquid form of PRF on woven and lamellar bone formation using circularly polarized light microscopy. Evaluation of birefringence of

collagen is a specific instrument to differentiate lamellar from woven bone.

Conflict of Interest

The authors have no conflict of interest to declare.

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