

Silver Impregnated Platelet-Rich Fibrin as a Barrier Membrane

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Abstract: Platelet-rich fibrin (PRF) was developed to eliminate nonfactors from of platelet-rich plasma to be used as a source of growth factor for tissue regeneration. PRF has been used as an autologous grafting material because of its ability to accelerate physiologic wound healing and new bone formation.

Aim: To compare mechanical, and histologic characteristics of the PRF membrane and Silver impregnated PRF.

Methodology: Venous blood was taken from the subjects for PRF preparation. Then, 2 normal PRF was prepared and for silver impregnated PRF.9 ml blood in addition to 1 ml of SNP suspension was poured into another tube and gently shook with hand to achieve a uniform 1% concentration. The tubes were centrifuged at 2700 rpm for 12 minutes.

1. The tensile test was done by using the Universal testing machine

2. The remaining pieces of the membranes were fixed in 10% formalin for 24 hours to be subjected to H&E staining and evaluated with the light microscope.

Results: Silver impregnated showed improved mechanical properties and dense fibrin network than PRF.

Conclusion: Silver impregnated PRF membrane demonstrated properties to be used as a barrier membrane for periodontal reconstruction.

Keywords: Platelet Rich Fibrin, Silver Nanoparticles

Introduction

Guided tissue regeneration (GTR) and guided bone regeneration (GBR) are surgical techniques that aim to reconstruct the damaged periodontal tissues which are lost due to periodontal lesions and to regain the alveolar bone, lost due to tooth extraction or periodontal disease¹. These methods employ various membranes to cover the bone and periodontal ligament and temporarily separate them from the epithelium and gingival connective tissue². Regenerative potential of platelets was first introduced in the 1970s, just when they were found to contain growth

factors responsible for increasing the collagen production, cell mitosis, blood vessels growth, and induction of cell differentiation³. The platelets were increasingly used in tissue regeneration over time. The platelet-rich fibrin can be used in various regenerative treatments to accelerate the healing and improve the regeneration procedure⁴. It can also be used as a scaffold in tissue engineering⁵. Nowadays, there are several techniques to obtain the high concentration of platelets, each of which results in a specific product that is unique in terms of biology and performance. These methods are generally classified into four groups based on their fibrin and leucocyte content: pure platelet-rich plasma (P-PRP), leucocyte- and platelet rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leucocyte-and platelet-rich fibrin

The chemical and physical properties of the membrane can influence the ultimate outcome of GBR and GTR⁷. The tensile strength of the tissue or the material which is sutured affects the success of suturing and the clinical results of wound healing⁸. Meanwhile, the membrane stiffness and presence of stiff material influence the distribution of mechanical forces over the surrounding tissues⁹. Generally, the better the mechanical properties of the membrane provide the better support for regenerative treatments.

The most frequent post operative complication of different regenerative techniques is the membrane exposure to the oral cavity, in which case, oral cavity microorganisms can colonize on the membrane and jeopardize the success of treatment¹⁰. It results in higher risk of infection and poor bone healing even in healthy individuals. Reinforcing the membranes' antimicrobial properties with inorganic materials can improve the treatment results. Inorganic antimicrobial materials have been more appreciated recently due to their safety and stability. One of the substances widely used today in various medical fields is

silver nanoparticle(SNP). SNP have the ability to anchor to the bacterial cell wall and penetrate in it, and Causes structural changes in the cell membrane affecting its permeability and cell death It causes formation of free radicals which lead to cell death.

It has been shown that these particles have high biocompatibility and also have favorable properties, including antimicrobial properties¹¹. Studies reported the effect of these materials on a wide spectrum of gram-negative and gram-positive bacteria as well as antibiotic-resistant species. Additionally, their antifungal and antiviral effects were proven¹⁰ the purpose of this study was to compare the mechanical, and histologic characteristics of the PRF membrane before and after the addition of SNPs.

Material and methods

A. PRF Preparation

Details of the study design and consent form was approved by the Ethical Committee .19 ml of blood was collected from healthy, nonsmoking volunteer aged 28 year (M) using VacutainerTM tubes and immediately centrifuged by a Medifuge centrifugation system at 2700rpm for 10 minutes. After the red thrombus (fraction of red blood cells) was eliminated from the PRF preparations, the resulting PRF was compressed with dry gauze for 15secondss.

B. Preparing SNP Suspension

To obtain a uniform suspension, 0.1 gm nanosilver powder with particles sized <100 nm (Sigma Aldrich; USA), along with 1 cc normal saline, was poured into the tube and sonicated at 200 W for 2 minutes in a sonicator device.

C. Silver nanoparticle impregnated PRF membrane

The remaining 9ml in addition to 1ml of SNP suspension was poured into another tube and gently shook with hand to achieve a uniform 1%concentration and then centrifuged by a Medifuge centrifugation system at 3000rpm for 10

minutes. After the red thrombus (fraction of red blood cells) was eliminated from the PRF preparations, the resulting SNP impregnated PRF was obtained

C. Histological processing

Both PRF were fixed in 10% neutralized formalin dehydrated, embedded in paraffin block sectioned sagittally. Sections were stained with Hematoxylin and Eosin (HE) for demonstration of nucleus and cytoplasm inclusions in specimens.

D. Physical properties

Tensile strength was measured by Universal Testing Machine. The "universal" part of the name reflects that it can perform many standard tensile and compression tests on materials, components, and structures. The specimen was placed in the machine between the grips and an extensometer. Once the machine was started it begins to apply an increasing load on specimen. Throughout the tests the control system and its associated software recorded the load and extension or compression of the specimen.

E. Accelerated degradation -In Vitro test

Two types of PRF membrane disks were freshly prepared and were inserted in 24-well plates and incubated in a CO₂ incubator with Hank's balanced salt solution (HBSS) supplemented with human plasmin (2.1g/mL).¹ HBSS was added to each well (24-well plate) by 0.5 mL and changed every 2 days.

Results

Histological Characteristics Evaluating the microscopic sections, the silver nanoparticles were observed all over the membrane, but in the outer layers they were more densely attached to the fibrin strands compared with the inner layers. Precipitation of the SNPs was patchy in the outer layers and quite homogeneous in the inner layers. Moreover, the leukocytes were denser in the outer layers than in the inner layers.

Physical properties The tensile strength of SNP impregnated PRF was greater than PRF.

Degradation time The control PRF was degraded in a time dependent manner and virtually completely digested at 6 days of incubation. In contrast, initially PRF initially turned the HBSS cloudy (8days), but thereafter the SNP impregnated PRF did not show appreciable degradation for at least 15 days of incubation.

Discussion

In the present study, the tensile strength was significantly higher in the SNP impregnated PRF membranes than the PRF group. The membrane's mechanical properties were improved as a result of adding SNPs to the PRF and its array in the fibrin matrix. The microscopic assessment of the samples showed that the SNPs were more densely mixed with the fibrin strands in the outer layers than the inner layers. Furthermore, their precipitation was patchy in the outer layer but quite homogeneous in the inner layers. This may justify the improved mechanical properties of the PRF membrane impregnated by SNPs. Yet, further studies by an electron microscope are suggested to investigate more details.

It is generally thought that a barrier membrane should be preserved at the implantation site for 3–4 weeks to enhance periodontal tissue regeneration and integration.¹⁵ Among absorbable membranes, those made of synthetic polymers such as polyglycolic acid and polylactic acid copolymer, demonstrate a slow degradation rate (=12 months), whereas collagen-based membranes degrade faster and have been reported to remain stable for 16–38 weeks without significant degradation.¹⁶ However, non crosslinked collagen membranes lose their structural integrity in 7 days. Walker KA et al in vivo animal implantation study demonstrated that the PRF could degrade as fast as noncrosslinked collagen-based membranes.¹⁵

Therefore, it is possible that increased crosslinking density among individual fibrin fibers within a PRF could prolong the preservation of the PRF at the implantation site and allow it to serve as a more clinically optimal GTR membrane.

The SNPs are highly biocompatible and have favorable properties such as antimicrobial property¹¹. Despite the several proposed theories, controversy exists regarding the mechanism of action of SNPs on microbes¹⁴. Silver nano particle scanad here to the cell membrane of the bacteria andmakeitporous,whichconsequentlyhangesthepermeabilityofthecellmembraneandcausescelldeath¹³.

Table I-Tensile strength of PRF membranes

Types	Tensile strength
PRF	0.02 N/mm ²
SNPimpregnatedPRF	0.07N/mm ²

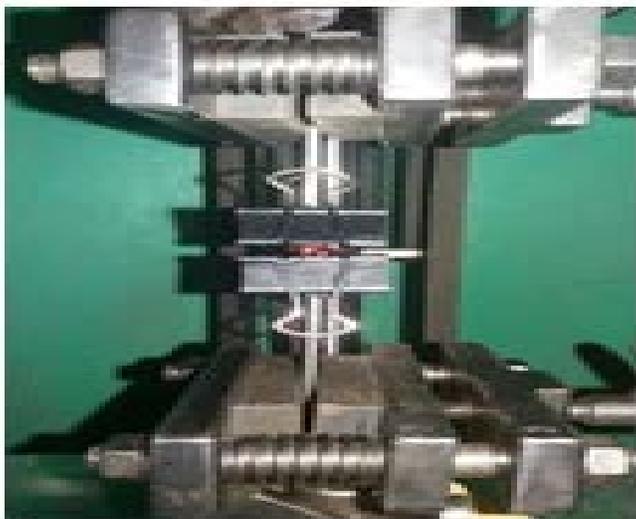


Figure I-Universal Tensile Machine

Conclusion: In the present study, SNPs impregnated PRF yielded a product which can help prevent the growth of a great family of bacteria (VGS) on the surgical sites and its consequences. This membrane not only has biological advantages, but also offers better mechanical properties including higher tensile strength, degradation time compared with the traditional membrane.

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