Original Article

Comparison of the Mechanical Properties of Platelet-Rich Fibrin and Ankaferd Blood Stopper-loaded Platelet-rich Fibrin

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Abstract

Background and Aim: Platelet-rich fibrin (PRF) can be named as a natural fibrin-based biomaterial favorable to increasing vascularization and able to guide epithelial cell migration to its surface. The membrane has a significant positive effect on protecting open wounds and accelerating healing. Similar to PRF Ankaferd Blood Stopper (ABS) also has positive effects on wound healing. The aim of this study was to detect if we can improve known physical properties of PRF combining with ABS. This idea was based on the known mechanism of ABS in forming protein network without damaging any blood cells. **Materials and Methods:** A total of 25 adult rabbits used for collecting 5–7 ml of blood passively with the help of winged blood collection needle to the test tube. Collected samples were centrifuged at 3000 rpm for 10 min. Two similar samples obtained from each animal and one of the samples was placed in 20% ABS 80% saline solution for 5 min. Mechanical properties of the membrane samples were measured using Universal Testing Machine. **Results:** There is the statistically significant difference between PRF and ABS added PRF in elongation/mm (dL) and elongation/% at break values. Maximum force (fMax) and modulus values did not show any statistically significant differences. **Conclusion:** ABS loaded PRF causes better physical properties. This combination seems to exhibit superior performance when used as a membrane barrier solely. Advanced studies can be done on biological properties of ABS loaded PRF, especially on tissue healing.

Keywords: *Ankaferd bloodstopper, mechanical properties, platelet-rich fibrin, wound healing*

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Introduction

There are numerous studies about accelerating wound healing. Platelet-rich fibrin (PRF) and platelet rich plasma (PRP) techniques are very popular as a reason of easy accessibility and successful reported studies. PRF technique does not need any gelling agent, which is different from PRP procedure.[1] It is only a centrifuged blood without any addition.[1] Blood sample is taken into a test tube, which does not contain any agent. This sample is centrifuged at 3000 revolutions/min (rpm) for 10 min.[1] Due to the absence of anticoagulant agent in the test tube, platelets come into contact to test tube wall, activating the coagulation cascades. Elapsed time for collecting blood and centrifugation is the main factor for success.[1] During this procedure, PRF polymerizes slowly with the help of thrombin.[1] Fibrin network is formed as a result of the transformation of soluble fibrinogen into insoluble fibrin with the help of fibrin stabilization factor XIIIa.[2] Platelet-rich fibrin (PRF) formed a tetramolecular structure containing cytokines, platelets, and stem cells.[2] Simonpieri *et al*.[3] concluded in their article that clinical sciences need practical solutions; therefore, only inexpensive and efficient

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techniques like PRF will continue to develop in the future.

Ankaferd blood stopper (ABS) (Ankaferd Health Products Ltd., Istanbul, Turkey) is a standardized herbal extract that has been approved for external bleeding hemostasis. It is a final product of five different plants (Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and Urtica dioica).[4,5] Mechanism of the ABS depends on forming an encapsulated protein network as soon as touching blood. This network provides focal attractive points for blood cell aggregation. This mechanism works independently from coagulation factors and platelets.[5]

The aim of this study was to determine how PRF and ABS loaded PRF responds to the forces applied. Comparison of the mechanical characters of these two membranes is aimed.

Materials and Methods

This study was approved by the Local Ethics Committee of Animal Experiments of Kırıkkale University; the decision is dated 12.27.2016 and numbered 16/92.

A total of 25 adult (12 males and 13 females) New Zealand rabbits (12-month-old) weighing an average of 3000 g were used for the study. The experimental animals were housed in appropriate cages maintained at a temperature of 22°C ± 2°C and a 12-h light/dark cycle. They were transferred to the laboratory environment at least a week before surgery to provide adequate health conditions, protect them from infections, help them in adapting to the new environment, and control their general health conditions. The animals were fed with standard laboratory food and water. Each 1 was housed in a separate cage to ensure comfortable access to water and food, an adequate range of motion, and a stress-free environment.

A mold was designed to obtain samples identical in size, volume, and figure. Mold was done with orthodontic bands and acrylic. For easy handling by the tester, the mold was prepared in 20 mm × 5 mm.

Marginal ear vein was used for blood collection. The rabbit was restrained by one of the researchers with the help of piece of cloth. The ear was warmed by gentle stroking actions to dilate the vein for successful collection. Venipuncture area was cleaned with 70% isopropyl alcohol. The ear was stretched away from the animal before needle insertion. In case of failed blood collection repeated insertion was done at a proximal part of the vein. A volume of 5–7 ml blood was drained passively from the winged blood collection needle to the test tube. Bleeding was stopped by applying gentle pressure on venipuncture site with the help of gauze and fingers.

Collected samples were placed as soon as possible in a centrifugal machine at 3000 rpm for 10 min. After this, three layers formed: lower fraction consists of red blood cells, middle fraction fibrin clot, and the upper part acellular plasma. The middle layer was used to form a membrane. Fibrin clot was placed on the grid of the PRF box and gently compressed with the lid of the box and placed there for 10 min to let squeezing out the fluids to standardize the thickness of the membranes. Two similar samples were punched out from the prepared membrane with the help of the mold [Figure 1]. One of the samples placed in 20% ABS 80% Saline solution for 5 min. Finally, one pure PRF membrane and one ABS loaded membrane obtained from the same sample. This procedure was done to minimalize the differences in samples. Procedure repeated for all of the samples.

Tensile test

Mechanical properties of the membrane samples were performed using universal (Zwick/Roell Gruppe/Ulm/Germany) with crosshead speed of 50 mm/min and gauge length of 20 mm according to (UK) [Figure 2]. An average of 5 test results has been reported.

Electron microscope analysis

Surface morphology of the samples was examined by electron microscopy [Figure 3]. Samples were washed with PBS and fixed with 2.5% glutaraldehyde (in PBS) for 20 min and dehydrated in ethanol solutions of 50%, 70%, 80%, 90%, and 100% for 5 min each. Followed by drying with 100% hexamethyldisilazane (HMDS) (Sigma Aldrich) for 3 min; excess HMDS was removed and samples aerated overnight. They were mounted on stubs and sputter-coated with platinum. Morphology was examined with JEOL LV 5610 SEM operating at an acceleration voltage of 20KV.

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows version 23 software (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk test was used to evaluate the normality assumption. Data are presented as the mean ± standard deviation or median (min-max). Paired sample *t*-test and Wilcoxon signed-ranks test were used when appropriate. A value of $P < 0.05$ was considered statistically significant.

Results

Electron microscopy scans shows connections between ABS particles and fibrin matrix [Figure 3].

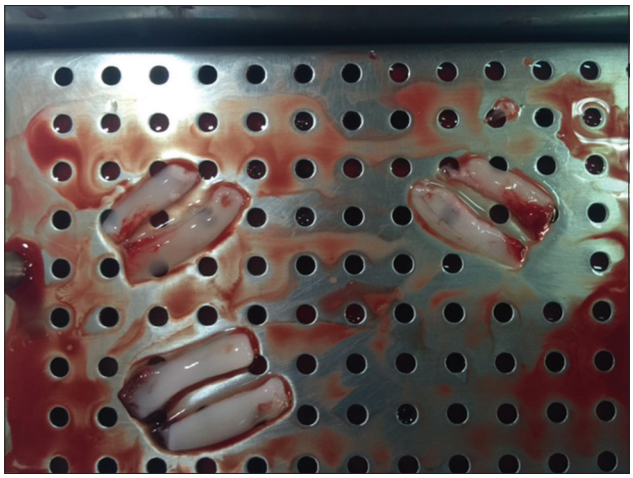

Figure 1: Two similar samples prepared by the help of the mold

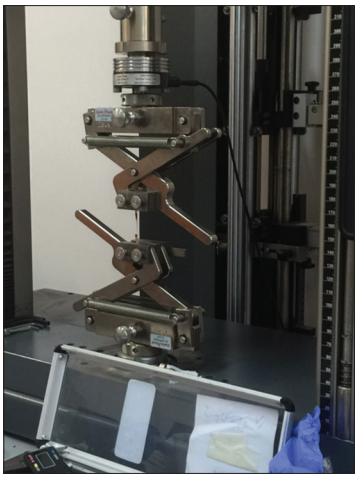

Figure 2: Test machine

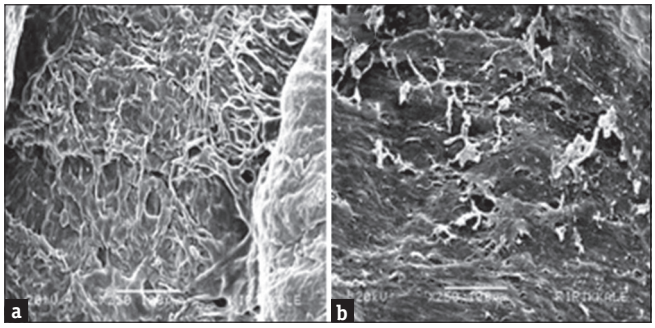

Figure 3: (a) The well-organized dense and mature fibrin matrix with a few cellular structures. (b) Ankaferd Hemostat (ankaferd blood stopper)-induced protein aggregates over and inside the fibrin matrix. Connections are seen between ankaferd blood stopper particles and fibrin matrix

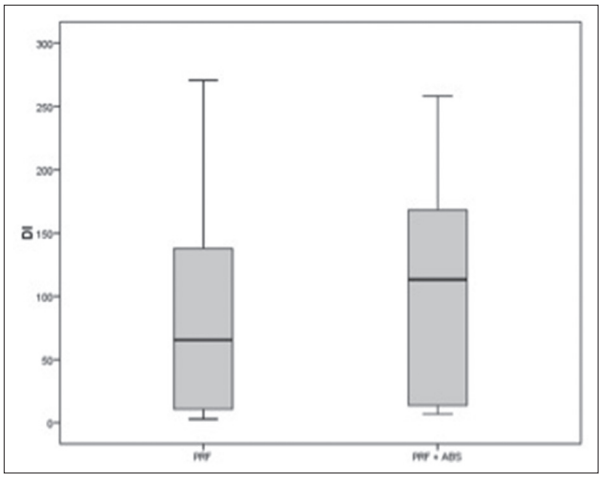

Figure 4: Result of dL control/experiment which is statistically significant (P = 0,024)

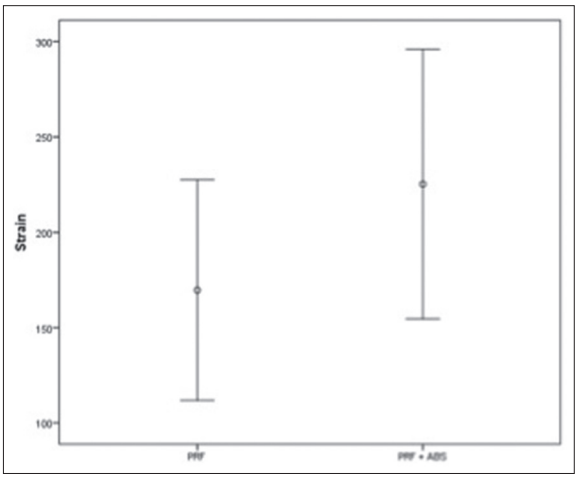

Figure 5: Result of strain which is statistically significant (P = 0,007)

Results showed that there is statistically significant difference between PRF and ABS added PRF in dL and elongation at break values [Figures 4 and 5]. The difference at stress at break and modulus was not significant [Table 1].

Table 1: Mean values of the results

	Mean±SD	Test statistics	*P*
fMax			
PRF	294.19±255.54	Z=−0.600	0.549
PRF + ABS	254.01±145.20		
dL (mm)			
PRF	81.175±77.16	Z=−2.257	0.024
PRF + ABS	96.996±85.21		
Modulus (kPa)			
PRF	0.26±0.24	Z=−0.243	0.808
PRF + ABS	0.25±0.20		
Elongation (%)			
PRF	169.69±57.93	t=−2.968	0.007
PRF + ABS	225.27±70.61		

PRF=Platelet rich fibrin; ABS=Ankaferd blood stopper; SD=Standard deviation; fMax=Maximum force

Discussion

There are numerous articles about PRF and ABS for

their clinical benefit. The idea about strengthening the fibrin network with ABS was based on the known mechanism of ABS about forming protein network. Both of the materials have great effects on tissue healing. The idea of the additive effect of both of them over tissue healing is the reason of this study.

High thrombin level in platelet-rich plasma (PRP) procedure causes tetramolecular or bilateral fibrin junctions which results in a rigid fibrin network. Low level of thrombin in PRF procedure causes trimolecular or equilateral fibrin branch junctions results in flexible, elastic, and strong membranes compared to PRP membranes.[1]

Khorshidi *et al*.[6] indicated that protocol used since years as 2700 rpm/12 min gives better-polymerized PRF which means stronger membranes than 3000 rpm/10 min protocol. Products of both procedures can be examined regarding mechanical quality. This study focuses on the advantages of adding ABS to PRF membrane; therefore, most preferred method is used in the study. For further studies, 2770 rpm/12 min can be studied and compared with the previous one.

A study performed on similarly aged rabbit blood to lessen possible differentiation related to age, sex, and systemic conditions.

PRF increases bone and soft tissue regeneration without any inflammatory reactions. Studies showed that using PRF alone or combination with grafts has positive effects on hemostasis, bone healing, and maturation.[2] Toffler *et al*.[7] indicated that PRF improves early wound closure, maturation for the bone grafts, and periodontal soft tissues. Besides PRF also ABS has positive effects on tissue healing, hemostasis, bone growth, and maturation. Aktas *et al*.[8] stated that ABS augmented immune reactivity of collagen Type 1, collagen Type 3, α-smooth muscle actin, fibronectin, 2 microglobulin, vascular endothelial growth factor, Cyclooxygenase-2, and mononuclear phagocyte marker during the first 7 days of soft-tissue healing of rat extraction sites. Combining PRF with ABS will improve biological properties besides physical ones. At this point, can the combination causes apoptosis of the cells or degradation of the cytokines should be evaluated. Haznedaroglu *et al*.'s[9] research is the first article about ABS and formed network. They showed that ABS forms a protein network and encapsulated cells in undamaged form. According to this and similar articles, this combination will show superior performance on the biological field. Research needed for biological behavior of the combination.

Most of the studies focused on mixture of graft materials such as freeze dried bone, β-tricalcium phosphate (β-TCP) allograft with PRF which accelerates wound healing. In addition decreases needed graft volume which helps in improved angiogenesis, revascularization and greater new bone formation.[7,10,11] Similar to these studies, Kim *et al*.[12] stated that PRF-mixed TCP showed more rapid bone healing than the rhBMP-2-coated TCP or the TCP-only control in their rabbit anterior sinus wall defect model. Mixing PRF with graft materials forms a "biological connector," which attracts stem cell to the center of the graft mixture and provides neo-angiogenesis.[13] Choukroun *et al*.[10] concluded in their PRF review that although cytokines trapped in PRF are gradually released and able to accelerate the cellular phenomenon, the structure of the fibrin network is the key element of all improved PRF healing processes.

In oral surgery procedures, PRF can be used as a resorbable membrane to prevent migration of soft-tissue cells during bone healing periods.[14] Also has the advantage of covering and stabilizing the graft particles. Gassling *et al*.[15] informed that PRF membrane is superior than a collagen membrane for using as a scaffold for the proliferation of periosteal cells *in vitro*. In a study, using PRF membrane and connective tissue graft following gingival recession operation compared and results were similar. PRF group shows more patient comfort and enhanced tissue healing.[16]

Gassling *et al*.[15] studied the coverage of the lateral sinus wall with collagen membrane and PRF and concluded that they have similar results for bone formation and residual bone substitute. Simonpieri *et al*.[13] published PRF grafting as a new method for grafting protocol. They augmented resorbed maxillary alveolar ridge and sinus lift with a mixture of freeze-dried bone graft, and PRF soaked in metronidazole. Authors used PRF as a membrane barrier between soft-tissue and hard-tissue and pointed out that PRF acted as fibrin bandages and quick closure of the wound was detected. As a result, they removed sutures on the day of three. PRF can easily be used both for particulate graft stabilization and guided tissue regeneration. There are numerous published successful case reports.[7]

PRF membrane degrades rapidly which is a short time corresponding with bone healing period.[2] Higher mechanical properties for membranes that are using for guided tissue regeneration is desired a feature. Kawase *et al*.[17] suggested heat compression of PRF membrane, which reduces the porosity and surface area results in delayed degradation up to 4 weeks. This study shows ABS improves physical properties of PRF. Electron microcopy images show new network formations between fibrin matrix and ABS molecules that may reduce porosity similar to Kawase's study. Advanced

studies can be planned for searching biological properties like degradation time.

Another area of using PRF is sinus lifting procedures. There are various literature studies that PRF is using as graft material or membrane. Mazor *et al*.[18] studied using PRF as a sole grafting material at a sinus lift procedure with simultaneous implant placement and concluded that using PRF which is an inexpensive and simple method, resulted with a high volume of natural regenerated bone. Toffler *et al*.[7] informed that they are using PRF membranes as membrane insurance for a possible undetected perforation during lateral window technique for sinus lifting procedure. Choukroun *et al*.[10] presented a patient which has a sinus perforation occurred in lateral wall sinus lift technique and treated successfully by PRF membrane in their PRF review reports. They also propose using PRF in Sinus lift procedure with a mixture of the graft material and under the incision line for accelerating tissue healing.[10] Similar to sinus cavity, PRF can be used after enucleation of an odontogenic cyst and promotes physiologic healing phenomenon. Choukroun *et al*.[10] emphasized that as PRF fibrin matrix is better organized, it can more efficiently direct stem cell harnessing and the healing program. Cystic cavity heals within 2 months instead of 6–12 months which is required physiologically.

The mold was prepared as an idea gotten from the Khorshidi *et al*.'s[6] article. The mold was prepared in a rectangular shape instead of dog bone shape to make preparation easier to produce and repeat.

Considering these findings, PRF can be named as a natural fibrin-based biomaterial favorable to increasing vascularization and able to guide epithelial cell migration to its surface. The membrane has a significant positive effect on protecting open wounds and accelerating healing. Furthermore, containing leukocytes and promoting their migration seems handy for using infected wounds.[10] Improving the physical properties of the fibrin matrix, like delayed degradation, can help for better clinical results.

Conclusion

The study shows that mechanical properties of ABS loaded PRF causes better physical properties. Increased strength seems to be due to the additional network over fibrin meshes. Solution percentage of ABS can be determined with different concentrations for the best result. Advanced studies can be done on biological properties, especially on tissue healing.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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