ORIGINAL RESEARCH ARTICLE

COMPARISON OF BLOOD CLOT ADHESION TO CONDITIONED ROOT SURFACE TREATED WITH PLATELET RICH PLASMA AND PLATELET RICH FIBRIN: A SCANNING ELECTRON MICROSCOPE STUDY

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ABSTRACT

Objective: To compare the blood clot adhesion to citric acid conditioned root surface treated with platelet rich plasma (PRP) and platelet rich fibrin (PRF).

Materials and Methods: 120 root specimens prepared from 60 extracted teeth were scaled and planed. They were then randomly allocated to 4 treatment groups, in which Group I samples were treated with only saline, Group II samples with citric acid and human blood, Group III samples with citric acid, PRP and human blood and Group IV samples with citric acid, PRF and human blood. All the samples were prepared for SEM analysis.

Photomicrographs were obtained using Scanning Electron Microscope under the magnification of 1000x, 2000x and 3000x to study the presence of blood cells and type of fibrin meshwork. The photomicrographs were examined by an independent examiner who was unaware of the study design and were scored using Blood Elements Adhesion Index (BEAI). The results were statistically analyzed using Chi-Square Test and Fisher's Exact test.

Results: Group I comprised of specimen showed no fibrin network and blood cells with $p<0.05$. Group II treated with Blood clot showed dense fibrin network with trapped cells (0%) & moderate fibrin network (50%) with $p<0.05$. Group III treated with PRP clot showed dense fibrin network with trapped cells (10%) & moderate fibrin network (80%) with $p<0.05$. Group IV treated with PRF clot showed dense fibrin network with trapped cells (80%). Conditioned root specimens treated with PRF clot were positively associated with dense blood cells and fibrin with $p<0.05$.

Conclusion: The adhesion of PRF clot was significantly better to citric acid conditioned root surface when compared to citric acid conditioned root surface treated with human blood alone and with human blood along with PRP.

Keywords: Citric Acid Root Condition; Human Blood; Plasma rich fibrin; Platelet rich plasma; Regeneration; Scanning Electron Microscope.

INTRODUCTION

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by a group of specific micro-organisms, resulting in destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both. This inflammatory disease of the supporting tissues leads to some crucial changes in the root surface of the teeth. Microorganisms associated toxins adsorbed to dental surface may promote periodontal breakdown through inflammatory

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response.[1] Bacterial products such as endotoxins have been detected in the cementum wall of periodontal pockets which have a cytotoxic effect on host cells.[2] Periodontal therapy involves scaling and root planning which is aimed at removing bacterial deposits and by-products from tooth surfaces. Nevertheless, this procedure produces a smear layer formed by residues of calculus, biofilm, contaminated cementum and dentin and bacterial by-products. Such surfaces may represent a poor substrate for plasma protein adsorption and subsequent fibrin adhesion[1]. Root conditioning agents have been developed to remove this smear layer and expose dentinal matrix which may favor formation of new cementum, periodontal ligament and bone by facilitating periodontal ligament cells to attach to the root surface.[1] Root surface conditioning with citric acid include a number of biological effects that may support periodontal wound healing and regeneration, as exposure of the dentin or cementum collagen matrix providing anchorage for the fibrin clot and subsequently new collagen fibrils.[2]

Platelet-rich plasma (PRP) is a kind of natural source of autologous growth factors, and has been used successfully for regenerative purposes, including that of the periodontal ligament.[1]

Platelet-rich fibrin (PRF), developed in France by Choukroun et al (2001), is a second generation platelet concentrate widely used to accelerate soft and hard tissue healing. It is a strictly autologous fibrin matrix containing a large quantity of platelet and leukocyte cytokines.[1]

Better adhesion & adsorption of blood cells & a formation of dense fibrin structure was observed following application of PRP on conditioned root surfaces.[3-5]

Studies have shown that PRF has favoured soft and hard tissue regeneration in periodontal defects.[3-6] One of the mechanisms of regeneration could be based on the adhesion of blood elements to PRF on the root surface during the initial phases of healing.[1,7]

Limited literature is available regarding second generation PRF facilitating blood cell adhesion on root surface which optimizes periodontal healing and regeneration.

Hence, the present study has been conducted to evaluate and compare the adhesion of blood clot to citric acid conditioned root surfaces treated with PRP and PRF.

MATERIAL AND METHODS

- **STUDY DESIGN:**

  An in vitro SEM study was conducted to assess the comparison of blood clot adhesion to conditioned root surface treated with platelet rich plasma and platelet rich fibrin.

- **INCLUSION CRITERIA:**

1. Patient age $\geq$ 30 years
2. Grade III mobility
3. Teeth extracted under L.A in non-traumatic method
4. Caries-free roots

- **EXUCLUSION CRITERIA:**

1. Patients who were under antibiotic therapy in last three months
2. Patients who underwent periodontal debridement in last six months

3. Patients who are current or former smokers

- **PREPRATION OF SAMPLES**
  - Extracted teeth were were cleaned in distilled water for 5 min and stored in 4% Phosphate Buffered saline (PBS), pH 7.4, at 370^0C until specimens were prepared.
  - Root specimens were prepared using a high speed diamond disc. This was done by drilling two parallel grooves on the proximal root surface of each tooth (mesial and distal): one at CEJ and other at approximately 4mm apical to the first groove. Then the area between the two grooves was scaled and planed with Gracey curettes 1-2 and 5-6 (HU-FRIEDY) to remove necrotic cementum until a hard smooth surface was achieved. Then using a diamond disc the roots were crosscut in the first groove, separating them from the crown. Then all roots were immediately cut lengthwise in the buccolingual orientation and then in the mesiodistal orientation as the apical groove were reached. Two root blocks approximately of 4x3mm were obtained from each tooth (mesial and distal).Thus a total of 120 specimens were obtained from 60 teeth. The specimens from each tooth were kept in bottle containing PBS.

- **PREPARATION OF PRP**
  To prepare platelet rich plasma 10 ml of blood was drawn from the antecubital vein of healthy volunteers. The blood was mixed with 1 ml anticoagulant thrombin and centrifuged. The centrifugation process consists of separation spin and concentration spin. For the separation spin, the blood was centrifuged at 1220 rpm for 15 minutes to separate the red blood cells from the rest of the whole blood (white blood cells, platelets, and plasma). Platelets and white blood cells formed the upper layer of the preparation, and the red blood cell fraction the lower layer. The upper layer and the top 1－2 mm of the RBC fraction, which are rich in newly synthesized platelets, was collected, and then centrifuged at 3600 rpm for another 15 minutes for concentration spin. The concentration spin results in the separation and compaction of the platelets, white blood cells, and a small number of residual red blood cells from the plasma. Most of the upper platelet-poor plasma (PPP) layer was aspirated away, and the remaining small volume of plasma was used for resuspending the concentrated platelets, a small number of red blood cells and white blood cells to develop the PRP

- **PREPARATION OF PRF**

A blood sample was taken without anticoagulant in 10ml tubes and was immediately centrifuged at 3000 rpm for 10 minutes. A fibrin clot was then obtained in the middle of the tube, just between the red corpuscles and PRP at the bottom and acellular plasma at the top. By driving out the fluids trapped in the fibrin matrix, a very resistant autologous fibrin was obtained.

The specimens were then randomly assigned to following Groups (30 specimens/ Group):

- *Group I (control):* saline
- *Group II:* Citric acid +blood
- *Group III:* Citric acid + blood + PRP
- *Group IV:* Citric acid + blood +PRF

Application of Saline

Group I samples were treated with normal saline for three minutes.[1]

Application of Citric Acid

Groups II, III and IV were treated with 25 % citric acid pH 1.0 by placing cotton pellets soaked with the acid and changing the solution every 30 seconds for a total period of 3 minutes.

PRP Application

The PRP was aspirated with a pipette and was applied to group III samples, after citric acid application, using a soft brush by leaving a uniform layer on the sample.

PRF Application

PRF was withdrawn from the test tube with help of tweezers and a fine membrane is prepared[10] and, after citric acid application, was applied to group IV samples with tweezers.

Blood Drop Application

Fresh blood was obtained from a healthy volunteer and a drop is placed on each prepared sample. The blood was allowed to clot for 20 minutes. After this period, samples were washed three times for 5 minutes in PBS, under soft agitation (60 rpm).

Preparation of Blocks

Using diamond discs, the samples were crosscut in the prepared grooves to separate them from the crown and obtain root blocks of approximately 5X5 mm dimensions.

Preparation of samples for SEM examination

The blocks were fixed in formaldehyde 1% solution in PBS for 15 minutes and washed three times for 5 minutes in PBS. After this, samples were incubated for 10 minutes in 0.02 M glycine solution in PBS and were washed as described earlier. Subsequently, samples were fixed in glutaraldehyde 2.5% solution in PBS for 30 minutes and washed as described earlier. Then they were dehydrated by immersion in ethanol concentrations of 25, 50, 75 and 95% for 10 minutes each and were washed three times for 10 minutes each in 100% ethanol. The dehydration process was finished in a critical point device. Finally, samples were mounted on metallic holders for sputter coating with 99.99% pure gold in sputtering machine.

SEM Examination

Photomicrographs were made of the centre area of each sample. Samples that received blood application were evaluated based on Blood Elements Adhesion Index {BEAI} under 1000x, 2000x, 3000x magnifications. Evaluation was performed by a trained and calibrated examiner.

Each photomicrograph was evaluated three times in intervals of at least 7 days. The score attributed to each sample was the most prevalent score of the three evaluations.

Blood Elements Adhesion Index (BEAI)[8]

0: Absence of fibrin network and blood cells
1: Scarcely distributed fibrin network and /or blood cells.
2: Moderate number of blood cells and/or thin fibrin network with poor interlacing.
3: Dense fibrin network with rich interlacing and presence of blood cells.

RESULT

To evaluate and compare the efficacy of clot adhesion on root surface of extracted teeth treated with citric acid alone and combination of citric acid along with PRP and PRF gel. Sixty periodontally diseased teeth indicated for extraction were obtained from study subjects with probing pocket depth & clinical attachment loss > 6 mm. Following local infiltration anesthesia with 2% Lignocaine with 1:100000 adrenaline probing pocket depth & clinical attachment level were measured to the nearest 1mm using UNC 15 probe. Teeth were then extracted as atraumatically as possible to prevent damage to the tooth surface. They were cleaned in distilled water and stored in 4% Phosphate Buffered saline (PBS), pH 7.4, at 370C until specimens were prepared.

Then root specimens were prepared using a high speed cylindrical bur under copious irrigation. This was done by drilling two parallel grooves on the proximal root surface of each tooth (mesial and distal): one at CEJ and other at approximately 4mm apical to the first groove. Then the area between the two grooves was scaled and planed with Gracey curettes 1-2 and 5-6 (HU-FRIEDY) to remove necrotic cementum until a hard smooth surface was achieved. Then using a diamond disk the roots were crosscut in the first groove, separating them from the crown. Then all roots were immediately cut lengthwise in the buccolingual orientation and then in the mesiodistal orientation as the apical groove were reached. Two root blocks approximately of 4x3mm were obtained from each tooth (mesial and distal).Thus a total of 120 specimens were obtained from 60 teeth. The specimens from each tooth were kept in bottle containing PBS.

The specimens were examined using SEM. The photomicrographs were scored using Blood Elements Adhesion Index (BEAI)

Comparative SEM analysis between the groups for Blood Element Adhesion Index

Group I (n=30): The group I comprised of controlled specimen in which zero specimens (0%) presented a score of 1, Zero specimens (0%) presented a score of 2 and zero (0%) specimens scored 3. All of the specimens scored 0 (100%) indicating no blood elements within the study group. (Table I)(FIG.1)

Group II (n=30): The group consisted of conditioned root specimens treated with Citric acid +blood. Fifteen specimens (50%) presented a score of 1, fifteen specimens (50%) presented a score of 2 and none (0%) specimens scored 3. None of the specimens scored 0 (0%). Moderate fibrin network was positively associated with Group II indicating that conditioning significantly improved the adhesion of the clot. (TableIII)(FIG.2)

Group III (n=30): The group consisted of conditioned root specimens treated with Citric acid +PRP+blood Three specimen (10%) presented a score of 1, twenty four specimens (80%) presented a score of 2 and three (10%) specimens scored 3. None of the specimens scored 0 (0%). Moderate and dense fibrin network was positively associated with Group II indicating that conditioning significantly improved the adhesion of the clot. (Table IV)(FIG.3)

Group IV (n=30): The group consisted of conditioned root specimens treated with

PRF clot. Three specimens (30%) presented a score of 2, while the remaining seven specimens (70%) scored 3. None of the specimens scored 0 (0%) or 1. Most of the specimens showed dense fibrin network with trapped blood cells indicating that conditioning of the root surface significantly improved the adhesion of PRF clot. (Table I) Comparing the four groups it could be inferred that severe to dense fibrin was positively associated with Group II and Group IV (p=0.191) (TableVI)(FIG.4)

DISCUSSION

Periodontitis is the most common form of destructive periodontal disease. Periodontitis affected root surfaces are hypermineralized and contaminated with cytotoxic and other biologically active substances,[2] such surfaces are not biocompatible and represent a poor substrate for plasma protein adsorption and subsequent fibrin adhesion. It has been suggested that uninterrupted adherence of the fibrin clot to the root surface is necessary to prevent the down growth of gingival epithelium.[9] Conditioning of the root may favor clot stabilization in early stages of periodontal wound healing to favor periodontal regeneration.[2]

The fibrin structure of the clot is important in the wound strength especially in early stages of healing [9] when it can be disrupted by forces acting on the wound. It is possible that a PRF clot formed by natural polymerization process[8] with large number of platelets and GF entrapped in the flexible fibrin strands may show better adherence & adsorption. The thick dense fibrin of the PRF clot may serve as a biologic construct facilitating the adhesion of the flap to the tooth, enhancing the proliferation and migration of cells from the periodontal ligament in support of periodontal regeneration, or support differentiation of mesenchymal stem cells into periodontal ligament cell phenotype.[10,11]

Present study therefore is an attempt to compare blood clot adhesion to conditioned root surface treated with platelet rich plasma and platelet rich fibrin. This was an in vitro comparative SEM study. Sixty periodontally invoved extracted teeth were used. Teeth from smokers or teeth with restorations extending below CEJ which could interfere with the preparation of root specimens or influence the adsorption of the blood elements to the root were excluded. The roots were sectioned & prepared.[12] the specimens were scaled & root planed to remove the endotoxins & bacteria that could inhibit the adhesion & adsorption of the blood elements to the root surface. The specimens were then randomly segregated into four treatment groups. Specimens in Group II, III & IV were conditioned with citric acid 25% (pH 1.0) [13,14] prior to application of HB, PRP or PRF clot respectively.

It is not possible to decontaminate a periodontitis-affected root surface completely by mechanical means alone.[15] Scaling and root planing procedures produce a 2.15 μm thick smear layer of microcrystalline debris which is intimately associated with the root surface and is virtually removed only by demineralizing agents.[15] Furthermore, the smear layer is resistant to rinsing, but may be removed with agents such as acids.[15] Hence, the critical step is to make the root surface compatible to establish a more suitable environment for periodontal regeneration.[16] In vivo histological studies

have shown compromised wound healing at root surfaces subjected to root instrumentation as a stand-alone protocol[17,18,19] while other studies have found no beneficial effects of such treatment.[20,21]

Root conditioning protocol used was as described by earlier author. It was applied using a cotton pellet dipped in freshly prepared 25% citric acid solution (pH-1.0)[22,23,13] prior to application of HB, PRP or PRF clot respectively. The total time of application was about 3 minutes with changing the solution every 30 seconds. Citric acid has been shown to be more effective than other root conditioning agents.[16,24] Questions have been raised about the anti-coagulant effect of citric acid. However, repeated rinsing with PBS removes the traces of CA & hence does not affect the polymerization of fibrin & its attachment to the root.[25] On the other hand, EDTA being a strong anti-coagulant may have adverse effects on blood clot formation, adhesion and stabilization.[24,12,13,15] Tetracycline hydrochloride has substantivity & may be difficult to remove from the root surface.[25,26]

10 ml of venous blood was drawn from healthy volunteers, for preparation of PRF. Patients with bleeding disorders & hematologic abnormalities were excluded as hematologic counts of the initial blood harvest have a significant bearing on composition of the PRF clot.[27] A number of protocols both manual & fully automated have been proposed for preparation of Platelet concentrates.[28] Although the standard method of preparation of PRF is the one described by Choukroun[10], the PRF in the present study was prepared using a tabletop centrifuge operating at 3000 rpm for 10 minutes. HB, PRP or PRF clot were applied to the roots which were then placed in humidifier at 37 ^{0} C for 20 minutes to facilitate adhesion of the clot to the root surface.

Following conditioning & clot adhesion five specimens from each group were rinsed with PBS on a rotary shaker & were designated as agitated while the other five specimens were designated as non-agitated to assess whether the forces produced by the shaker disrupted the adhesion of the clot.[29,30,31] The specimens were then prepared for SEM examination. SEM is a tool that gives three dimensional pictures at ultra-structural level.[27] Standardized photomicrographs were obtained at 1000x, 2000x, 3000x magnifications. Morphology of the treated root specimens was assessed based on defined criteria[12,13,32] by an independent examiner who was unaware of the study.

Comparative SEM analysis between the groups showed that blood elements adhered and adsorbed to the root surface irrespective of the type of clot and the nature of the root surface. However there was variability in the adsorption & adhesion of blood elements scores within each group.

No blood cells and fibrin network was observed in controlled group (Group I) whereas moderate the conditioned root surfaces treated with HB (GroupII) were predominated by moderate to dense fibrin network with trapped cells (40%). The group III which comprised of conditioned root specimens treated with PRP clot showed scarce to moderately distributed fibrin network with trapped cells (50% each) , indicating that the physical and chemical characterstic of root surface had influence on the adherence of clot. A phenomenon of similar kind has been observed in implant studies when platelet

concentrates were used to modify the surface of implant to improve osseointegration.[33]

Most of the conditioned root specimens (Group IV) treated with PRF clot showed dense fibrin network with trapped cells (70%). It was observed that the conditioned root specimens treated with HB or PRF clot were positively associated with dense blood cells and fibrin ($p \leq 0.0001$). Comparative studies for natural blood clot & PRF are lacking. However, studies have compared adhesion of HB & PRP to root surfaces treated with different conditioning agents.[10] Our results are in agreement with earlier studies in which PRP application on conditioned root surfaces showed a moderate fibrin network.

Comparative SEM analysis among groups treated with both HB and PRF for Blood Element Adhesion Index showed that conditioning of the root surface had a significant effect on the adhesion & adsorption of PRF clot ($p \leq 0.0001$).

A number of factors influence the interaction of blood with foreign substance. Composition and organization of initial protein layer mediates platelet interaction and determines long term effects. Heparin treatment of the root surface compromises the attachment of clot[34] while fibronectin of all plasma proteins strongly adsorbs to collagen.[35] The amount of fibronectin per PRF clot is about 1µg at the end of 20 minutes increasing upto 5.3µg at the end of one hour.[36] Fibronectin from the exudate of PRF clot can saturate the root surface facilitating the cell adhesion.[35,37] Interaction of polymerizing fibrin with platelets is accompanied by loss of serotonin and ADP from dense granules of the platelets.[39] This in turn enhances aggregation and release of the granular content of the platelets setting the stage for accelerated healing.[37] The surface demineralization by citric acid causes exposure of collagen fibers increases the wettability of the root, which helps in adsorption of plasma proteins. The degree of adsorption in turn determines the subsequent events of wound healing. Well demineralized root surface with exposed collagen are thrombogenic.[40,41,42] In absence of collagen, the fibrin network only opposes but cannot get attached to the root.[43] In vivo histologic studies have shown that wound healing is enhanced at root surfaces subjected to root instrumentation followed by root surface demineralization.[44,45] Studies in well-defined animal models have provided evidence of a new connective tissue attachment rather than an epithelial attachment when the affected root surfaces had been exposed to demineralization following instrumentation.[46] Extensive studies have shown the effect of CA conditioning in forming & retaining a stable blood clot.[46,47,70,71] Blood elements imposed onto the root surface during surgery and at wound closure must establish an attachment that endures normal physiologic and other potentially rupturing forces acting on the tooth–gingival flap interface. This attachment must remain stable until such time as the interface has matured to sufficient tensile strength to offset any impact from functional or other forces.[43]

Statistically significant differences in the adhesion & adsorption of natural blood clot, PRP and PRF clot to the conditioned root surface were observed.

Theoretically the PRF clot may provide a significant benefit in subsequent stages of healing, as healing is a complex process.

PRF clot is a reservoir of GF & cytokines trapped in the matrix & released in a controlled manner for at least seven days. It has more number of leukocytes & hence is immune concentrate as well.[10] Dense flexible fibrin formed by natural progressive polymerization supports angiogenesis & provides scaffold for invading cells. SEM studies have shown that PRP enhanced the attachment & proliferation of human periodontal ligament cells to the conditioned root surfaces.[80] PRF has been shown to modulate cell proliferation in a cell type specific manner. PRF stimulates proliferation of OB, gingival fibroblasts and PL cells while suppressing epithelial cell growth.[48,49,50] PRF may also stimulate the formation of cementum.[50]

PRF when placed at the interface between the root and the bone serves two specific mechanisms: impregnation and induction. Root surface is impregnated with blood protein facilitating the establishment of the biologic linkage between two surfaces. The slow release of growth factors and cytokines from the platelet within the matrix triggers cell induction.[10] Thus PRF is an enriched blood clot that may efficiently direct the healing program. Based on the observations of this study it can be concluded that both natural blood clot and PRF were associated with moderate to dense fibrin formation on conditioned root surfaces. PRF clot adhered poorly to unconditioned root surfaces when compared to a natural blood clot. Therefore, root conditioning with citric acid may be considered when platelet concentrates are used in regenerative therapy.

CONCLUSION

An in vitro SEM study was undertaken to evaluate and compare the blood clot adhesion to citric acid conditioned root surface treated with human blood, platelet rich plasma and platelet rich fibrin with the following conclusions:

1. The adhesion of PRP clot was significantly better to citric acid conditioned root surface when compared to citric acid root conditioned root surface treated with human blood.
2. The adhesion of PRF clot was significantly better to citric acid conditioned root surface when compared to citric acid root conditioned root surface treated with human blood alone and with human blood along with PRP.
3. Conditioning influenced the adhesion of PRF clot and natural blood clot as indicated by the formation of a dense fibrin network and entrapment of blood cells within the fibrin matrix.
4. The study demonstrated that citric acid root conditioning followed by PRF application may favor blood cell adhesion on root surface which optimize periodontal healing.

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TABLES:

Table I: Comparison of BEAI scores between Group I, II, III, IVBEAI Scores

	Group I n=30	Group II n=30	Group III n=30	Group IV n=30
Absence of fibrin network and blood cells(0)	30(100%)	0	0	0
Scarcely distributed fibrin network and/or blood cells(1)	0	15 (50%)	3(10%)	0
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	0	15 (50%)	24(80 %)	6(20%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	0	3(10%)	24(80%)

Table II: Comparison of BEAI scores between Group I, II, III, IV

P value	<0.0001
Chi-Square	213.07

Table III: Comparison of BEAI scores between Group I & II

BEAI Scores	Group I n=30	Group II n=30
Absence of fibrin network and blood cells(0)	30(100%)	0
Scarcely distributed fibrin network and/or blood cells(1)	0	15 (50%)
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	0	15 (50%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	0

Table IV: Comparison of BEAI scores between Group I & II

P value	**<0.0001**
Chi-Square	**60**

Table V: Comparison of BEAI scores between Group I & III

BEAI SCORE	GROUP I	GROUP III
Absence of fibrin network and blood cells(0)	30(100%)	0
Scarcely distributed fibrin network and/or blood cells(1)	0	3(10%)
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	0	24(80%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	3(10%)

Table VI: Comparison of BEAI scores between Group I & III

P value	<0.0001
Chi-Square	60

Table VII: Comparison of BEAI scores between Group I & IV

BEAI Scores	Group I n=30	Group IV n=30
Absence of fibrin network and blood cells(0)	30(100%)	0
Scarcely distributed fibrin network and/or blood cells(1)	0	0
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	0	6(20%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	24(50%)

Table VIII: Comparison of BEAI scores between Group I & IV

P value	<0.0001
Chi-Square	60

Table IX: Comparison of BEAI scores between Group II & III

BEAI Scores	Group II n=30	Group III n=30
Absence of fibrin network and blood cells(0)	0	0
Scarcely distributed fibrin network and/or blood cells(1)	15 (50%)	3(10%)
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	15 (50%)	24(80%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	3(10%)

Table X: Comparison of BEAI scores between Group II & III

P value	0.045
Chi-Square	13.08

Table XI: Comparison of BEAI scores between Group II & IV

BEAI Scores	Group II n=30	Group IV n=30
Absence of fibrin network and blood cells(0)	0	0
Scarcely distributed fibrin network and/or blood cells(1)	15 (50%)	0
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	15 (50%)	6(20%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	0	24(50%)

Table XII: Comparison of BEAI scores between Group II & IV

P value	<0.0001
Chi-Square	42-86

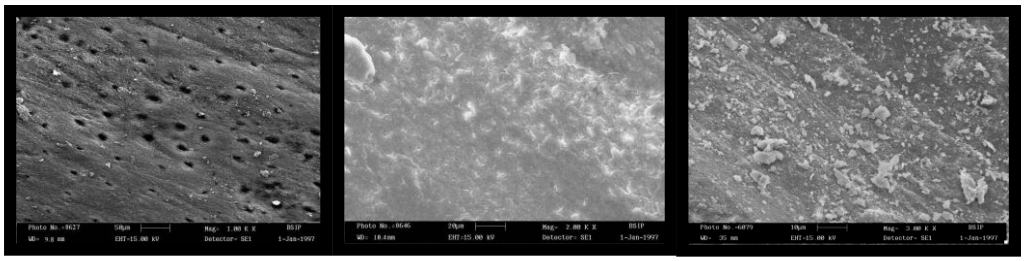
Table XIII: Comparison of BEAI scores between Group III & IV

BEAI Scores	Group III n=30	Group IV n=30
Absence of fibrin network and blood cells(0)	0	0
Scarcely distributed fibrin network and/or blood cells(1)	3(10%)	0
Moderate number of blood cells and thin fibrin network with poor interlacing(2)	24(80%)	6(20%)
Dense fibrin network with rich interlacing and presence of blood cells(3)	3(10%)	24(50%)

Table XIV: Comparison of BEAI scores between Group III & IV

P value	<0.0001
Chi-Square	30.18

FIGURES:

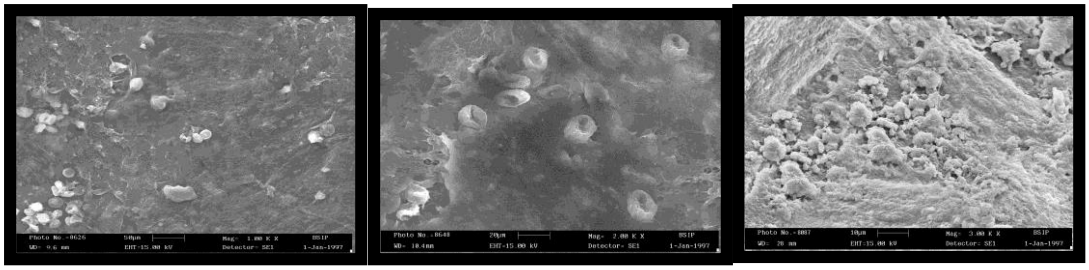


(A)1000x Absence of fibrin network and blood cells

(B) 2000x Absence of fibrin network and blood cells

(C) 3000x Absence of fibrin network and blood cells

Fig. 1 SEM photomicrographs of Group I - Control

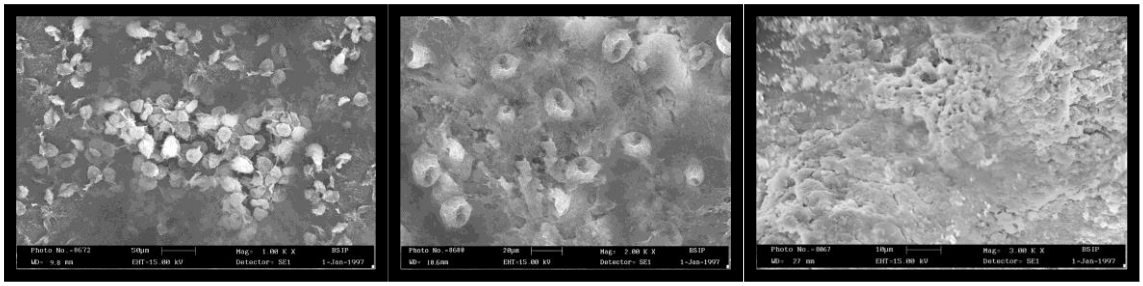


(A)1000x Scarcely distributed fibrin network and blood cells.

(B) 2000x Scarcely distributed fibrin network and /or blood cells

(C) 3000x Scarcely distributed fibrin network and blood cells.

Fig. 2 SEM photomicrographs of Group II- Citric Acid + Human Blood

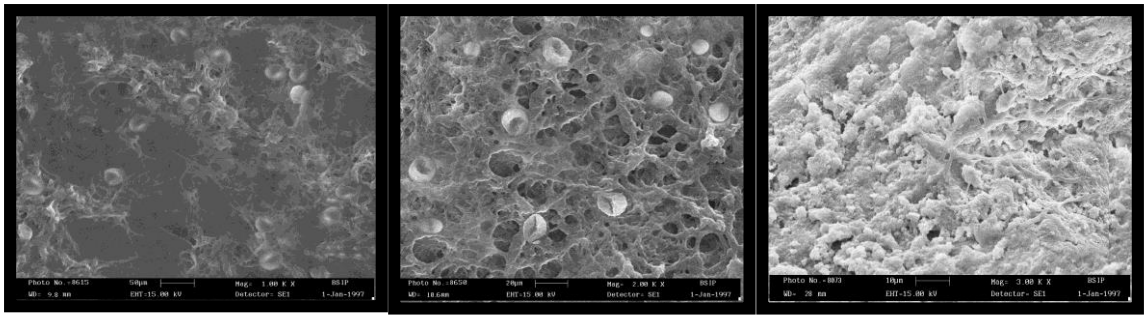


(A)1000X Moderate number of blood cells and thin fibrin network with poor interlacing

(B)2000x Moderate number of blood cells and thin fibrin network with poor interlacing

(C) 3000x Moderate number of blood cells and thin fibrin network with poor interlacing

Fig. 3 SEM photomicrographs of Group III - Citric Acid+ PRP +Human Blood



(A)1000x Dense fibrin network with rich interlacing and presence presence of blood cells.

(B) 2000x Dense fibrin network with rich interlacing and presence of blood cells.

(C) 3000x Dense fibrin network with rich interlacing and of blood cells.

Fig. 4 SEM photomicrographs of Group IV- Citric Acid+ PRF+ Human Blood