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# Comparison of Platelet-Rich Fibrin (PRF) Membranes Produced by Three Centrifuges using a Histological Scoring Methodology

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**ABSTRACT**

**Aims:** The aims of the current study were to show the differences and similarities between original Platelet-Rich Fibrin (PRF) clots produced by a specifically designed centrifuge for this purpose and those clots produced by other centrifuges using bright light microscopy.
**Materials and Methods:** The study included five human volunteers. From each volunteer, six 9 ml blood samples were collected (Total number= 30) and immediately centrifuged, each ten blood samples allocated to a centrifuge group (total of three), one of which was the original recommended centrifuge (Hettich). A set of six histological observations were made and considered as a histological scoring system for the purpose of comparison of different membranes.
**Results:** Out of the six observations, only two showed a significant difference between the three membranes produced namely density of inner fibrin zone density and cell border morphology.
**Conclusions**: Differences in clot membrane histological observations are due to the centrifuge characteristics, namely heat generation and vibrations. Within the limitations of the current study, any Platelet Rich Fibrin clot produced without respecting the original protocol should be termed Leucocyte Platelet-Rich Fibrin -like product and this term is preferred to be added to the recent global classification.
**Keywords:** Platelet- Rich Fibrin, PRF, Centrifuge.

## I. INTRODUCTION

Wound healing is completely dependent on the early mechanisms of hemostasis. The first tissue to react when an organism is wounded is the circulating tissue: the blood. The wound elicits a cascade of reactions leading to the sealing of the vascular breach with platelets aggregates which not only arrest the hemorrhage in the damaged tissue, but also prepare the forthcoming steps of tissue regeneration. Platelets deliver on the wounded sites a massive load of fibrinogen and enzymes, and also release huge amounts of various molecules, particularly growth factors. Platelet fibrinogen and circulating fibrinogen start to polymerize into a dense fibrin network in order to glue and close the wound with a solid wall [(1)]. The fibrin matrix is the final purpose of this complex cascade of reactions: the coagulation [(2)]. Platelets, leukocytes, fibrin matrix and many growth factors work together in synergy during the coagulation process, and many products logically tried to mimic these natural mechanisms in order to improve healing on a surgical site [(1, 3-6)].The use of blood derived products as surgical adjuvants to seal and stimulate wound healing is one of such trends.

A new family of platelet concentrates, which is neither fibrin glue [(7)] nor platelet- rich plasma (PRP) [(8)], appeared in France. This natural biomaterial was termed platelet-rich fibrin (PRF) and was developed by Choukroun's et al. in 2001 [(9)] for the specific use in oral and maxillofacial surgery.Choukroun's platelet-rich fibrin (PRF) is a second generation platelet concentrate defined as an autologous platelet-rich, leukocyte and fibrin biomaterial and can be termed as a super clot [(9-13)]. Compared to other platelet concentrates, this technique does not require any anticoagulants or bovine thrombin or any other gelling agent. The protocol for its preparation is very simple and inexpensive: blood is collected in 10-ml dry glass tubes or glass-coated plastic tubes without anticoagulant and immediately softly centrifuged at 3000 rpm (approximately 400**g**) for 10 minutes [(9)].At the end of centrifugation, three layers are formed in the tube: a red blood cell (RBC) base at the bottom, acellular plasma (platelet-poor plasma - PPP) as a supernatant, and a PRF clot in the middle (Dohan Ehrenfest et al., 2006b). This clot combines many healing and immunity promoters present in the initial blood harvest. It can be used directly as a clot or after compression as a strong membrane [(13)].The different uses are mentioned in the literature [(14-25)].

In order to highlight the need to respect the original protocol and material, or at least to define clearly any variations of the PRF protocol / material as a different protocol, it is suggested that changes in protocols and / materials may considerably affect the PRF clot content and architecture and must therefore be considered separately as a specific PRF-like product and not as the original PRF described in the literature. This dispute is very important, in order to

avoid creating confusing data in the literature that may affect the credibility of the PRF technique because of methods or materials. One important factor is the mechanical characteristics of a centrifuge used to produce that clot which may ultimately interfere with the quality and biological signature of the final PRF product [26](Dohan Ehrenfest, personal communication, 2014).

The current conducted study was an attempt to compare three PRF membranes produced by three centrifuges, the first being the original Hettich model centrifuge specifically designed for this purpose and two other table top centrifuges namely model 800-D and model 80-2 centrifuges. A bright light microscope methodology for PRF membrane comparison is shown.

## MATERIALS AND METHODS

**Sample**: Approval of study was from the scientific research committee / Department of Oral and Maxillofacial Surgery / Dental College / Mosul University / IRAQ in the period (January /2014). Five healthy male volunteers (dental colleagues) were enrolled with ages ranging from 30 to 40 years (mean = 35 years). Inclusion criteria were healthy non-smokers, no aspirin intake or any medication that could interfere with the blood coagulation process. The location settings were at the Department of Oral and Maxillofacial surgery / Mosul Dental College in the period (January / 2014). Both verbal and written consents were implicated.

**Methodology:** Blood collection procedures were performed in early morning sessions (8.30am-9.15 am); one volunteer a day for 5 consecutive days. This was to insure standardization. Prior to blood collection, blood pressure was measured using an aneroid manometer (China) and stethoscope (China) with cuff on left arm of volunteer. With the volunteer in a comfortable upright sitting position and area of needle insertion disinfected, six-9 ml blood samples were collected in glass containers (Jordan) to produce a total of thirty PRF clots. Allocation of blood samples to centrifuge assigned was randomly made (yet two consecutive samples for each centrifuge).The relative centrifuge force (r.c.f.) was standardized close to 400 **g** for all three centrifuges. For each section, and based on the centrifuge model to be used for the production of PRF clot, three groups were designed:

**Group Hettich:** The PRF clots produced by Hettich (original) model centrifuge / Germany (centrifuge spin of 3000 rpm for 10 minutes).

**Group 800-D**: The PRF clots produced by 800-D model centrifuge / China (centrifuge spin of 3000 rpm for 10 minutes).

**Group 80-2:** The PRF clots produced by 80-2 model centrifuge / China (centrifuge spin of 2700 rpm for 12 minutes).
At completion of each centrifuge spin, each clot was removed from its container and gently laid on a 2*2 piece of damp sterile gauze for two minutes. The clots were then placed in 10 % buffered neutral formalin (a separate 50 ml container) for 24 hours without any forcible compression. On the second day, the clots were dehydrated in an ascending series of alcohol concentrations (70%, 95% and 100%) for a one hour interval for each concentration and finally in 100% left overnight. On the third day, the clot specimens were then placed in toluene (one hour) before paraffin embedding [27]. The clot has now turned into a membrane. For sectioning, a series of twenty full length consecutive cuts were performed for each membrane with a thickness of 5 µm along the longitudinal axis of the (Fig 1, A) membrane and stained with Hematoxylin and Eosin (Fig 1, B). From these twenty serial cuts, five sections were randomly selected and subjected to bright light microscopical examination. The examination was conducted by two specialists. The examination of each specimen was based on six observations with score grading's for each (Table - 1) (Dohan Ehrenfest, 2013; personal communication and Haj-Qasim, 2014; personal communication).

**Table – 1: Histological observations and scoring for platelet-rich fibrin (PRF) membranes.**

| Observation | | Score | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 1 | Zone layers in the membrane | Well demarcated | Poorly demarcated | |
| 2 | Cell nests in the inner fibrin fiber zone | Inside | Outside | |
| 3 | Density of inner fibrin fiber zone | Very dense | Dense | Loose |
| 4 | Number of cell layers at border | More than 10 | Less than 10 | |
| 5 | Aggregation of cells in the cell layer zone | Heavy | Light | |
| 6 | Cell border morphology | Very clear | Unclear | Very unclear |

**Each observation selected was based on a certain criterion to be seen and as followed:**
**Zone layers in the membrane:** This observation was examined under low power (×40). The membrane was noticed to be composed of three zones; dense inner fibrin zone representing the majority of the membrane body; an outer thin zone of a loose network of fibrin fibers and a cellular zone attached to the loose fibrin fiber zone at the lower lateral and bottom area of specimen. A clear demarcation could be noticed between these three zones and was described as well (score 1) or poorly demarcated (score 2), yet attached to each other.

**Cell nests in the inner fibrin fiber zone:** This was examined under moderate power (×100) and was described as either inside (score 1) or outside (score 2).

**Density of inner fibrin mesh:** This was examined under high power (×400) and was based on the number of pores within the fibrin mesh as very dense with less than 25 pores (score 1), moderate density with pores between 25-50 (score 2) and loose network with pores more than 50 (score 3).

**Number of cell layers at border**: This observation was examined under high power (x400). The number of cell layers at the periphery of clot were counted as more than ten layers (score 1) or less than ten layers (score 2).

**Aggregation of cells in the cell layer zone:** This observation was examined under high power (×400) in the cellular zone of specimen and was based on the proximity of cells to each other as either heavy (score 1) or light (score 2).

**Cell border morphology**: This was examined under high power (x 400) and was based on clear cell border morphology as either **Score 1**: Very clear **Score 2**: Clear, **Score 3:** Very unclear.
For each observation evaluated, five areas in each specimen were selected and examined (Fig.1, B) and a score was given for each. The final mean score was considered for statistical analysis.

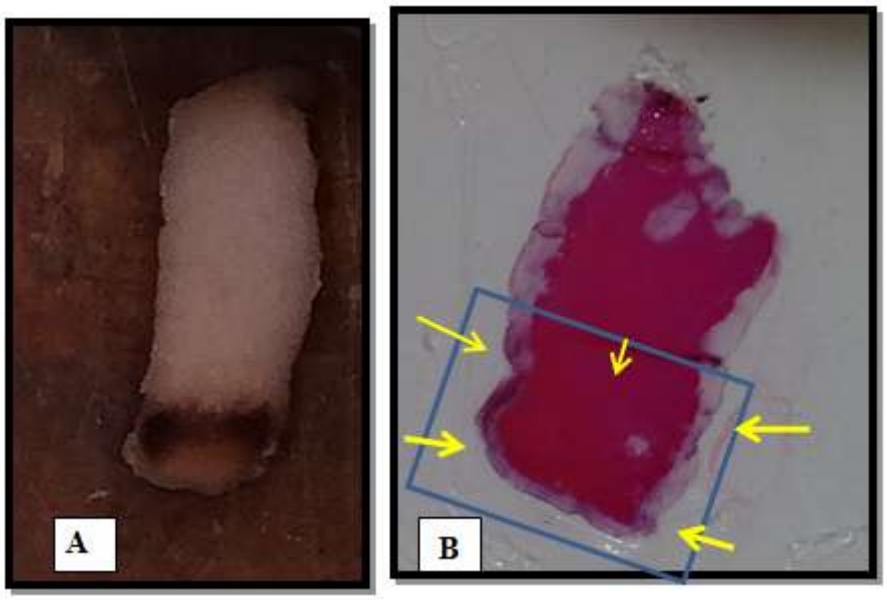


**Figure 1, (A) Serial cuts were made along the longitudinal axis of membrane (not in paraffin blocks for purpose of photography) (B) Staining of membrane with Hematoxylin and Eosin stain and areas for observation.**

**Statistical analysis:**
All statistical analysis was performed using commercially available statistical software program (SPSS Version 16.0; Chicago, IL, USA). The following tests were used:percentage, Pearson Chi –Square test and Mann-Whitney test. Significant statistical difference was set at $\mathbf{p} < 0.05$ and $\mathbf{p}<0.01$

## RESULTS

In PRF membranes for groups Hettich, 800-D and 80-2, under low power field magnification (x40), the following **general** features were seen: A fibrin matrix light pink in colour representing the main body of the membrane with heavy cellular aggregates appearing as dark blue at its lower periphery (junction between the fibrin and buffy coat). Between the fibrin network and cellular components, a loose area of fibrin network than its inner aspect was seen (Fig. 1,A,B). At moderate (x100) and high power (x400) magnification, the following was seen; a varying densely packed fibrin network with pores in between the fibres. The cellular components were mainly leukocytes represented by neutrophils with multi lobulated nuclei and lymphocytes with big nuclei filling almost the entire space of its cytoplasm.

The cytoplasm of these cells stained a darker pink than the adjacent light fibrin network and their nuclei stained as dark blue. Platelets and residual red blood cells also appeared light pink and although could be discriminated from adjacent fibrin network, were difficult to identify because of clumping (Fig.7).
In the histological scoring system designed for the study, the following was observed:

**Zone layers in the membrane**:

The highest percentage of membranes showing a well demarcated pattern **(**Fig. 2,A,B)in 80% ofmembrane specimens were in group Hettich and 20% poor demarcation followed by group 800-D with 60% of specimens showing well demarcated zones with 40% poor demarcation and in group 80-2; 50% well demarcation and 50% poor demarcation. The Pearson Chi-Square values showed no significant difference among centrifuge groups(Table - 2).

**Presence of cell nests in the inner fibrin zone**:

The percentage of cell nests in the inner fibrin zone in groups Hettich and 800-D were 20% while in group 80-2 was 10%. The majority of cellular population was outside that zone (in the cellular layer) which was reflected as 80% of cell nests outside in groups Hettich and 800-Dand 90% outside cell nests in group 80-2 (Fig 3,A,B).The Pearson Chi-Square values showed no significant difference among centrifuge groups in both study models (Table - 2).

**Density of the inner fibrin zone**:

In group Hettich membranes, very dense fibrin network was shownin(90%) of specimensand only 10%dense fibrin network and no loose fibrin network**.** In group 800-D,very dense fibrin network was shownin(10%)of specimens, 50%dense fibrin network and 40% loose fibrin network. In group 80-2, very dense fibrin network was shown in 20% of specimens, 50 % dense fibrin network and 30% loose fibrin network (Fig 4, A, B, C).The Pearson Chi-Square values showed a highly significant difference among the three centrifuge groups in regard to the density of the fibrin network with the highest percentage of very dense fibrin network in group Hettich for both study models followed by group 800-D and group 80-2 (Table -2). In comparison between centrifuge groups, the Mann-Whitney test results showed a highly significant difference in group Hettichfor both study models when comparedwithgroup's 800-D and 80-2 but no statistical difference between the latter two groups (Table -3).

**Number of cell layers at border**:

The percentage of more than 10 cell layers in groups Hettich, 800-D and 80-2 was 90%, 80% and 80% respectively and less than 10 cell layers in 10%, 20% and 20% of specimens respectively (Fig 5, A, B).The Pearson Chi-Square values showed no significant difference among centrifuge groups for both study models in this regard (Table 2).

**Aggregation of cells in the cell layer zone:**

In human PRF membranes, group Hettich showed 90% heavy aggregation and 10% light aggregation. In group 800-D, heavy aggregation of cells was observed in 70% and light cell aggregation in 30% of specimens. In group 80-2**,** the percentage of heavy cell aggregation was 80% and light aggregation in 20% of specimens (Fig 6, A, B).The Pearson Chi-Square values showed no significant difference among centrifuge groups in both study models in this regard (Table - 2).

**Cell border morphology:**

In human PRF membranes, group Hettich specimens showed 20% very clear cell wall morphology, 70% unclear cell wall morphology while 10% very unclear cell wall morphology with clumping into groups. In group 800-D specimens observations showed 10% very clear cell wall morphology, 20% unclear cell wall morphology while 70% very unclear cell wall morphology with clumping into groups. In group 80-2 specimens, observations showed 10% very clear cell wall morphology, 10% unclear cell wall morphology while 80% very unclear cell wall morphology with clumping into groups (Fig 7, A, B, C), (Table -2).

The Pearson Chi-Square values showed a highly significant difference among centrifuge groups (Table-2).In this regard, comparison between centrifuge groups in both study models, the Mann-Whitney test results showed a significant difference between groups Hettich and 800-D, highly significant difference between groups Hettich and 80-2 in human PRF membranes but no significant difference between group's 800-D and 80-2.In sheep PRF membranes, a significant difference between groups Hettich and 800-D, groups Hettich and 80-2was also disclosed but no significant difference betweengroup's 800-D and 80-2(Table -3).

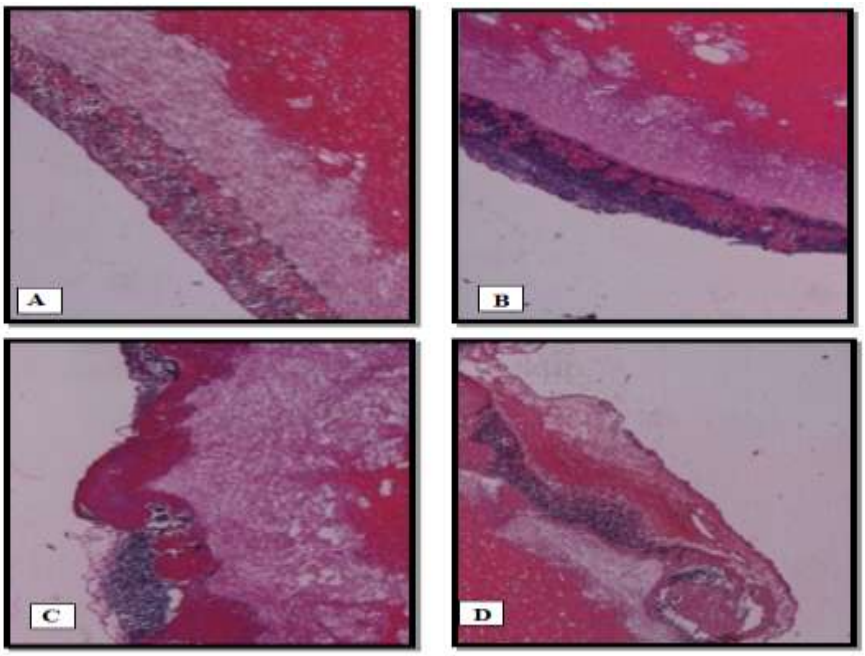


**Figure 2: Arrangement of zone layers: Score 1(A, B) Well demarcated; Score 2 (C, D) Poorly demarcated.**

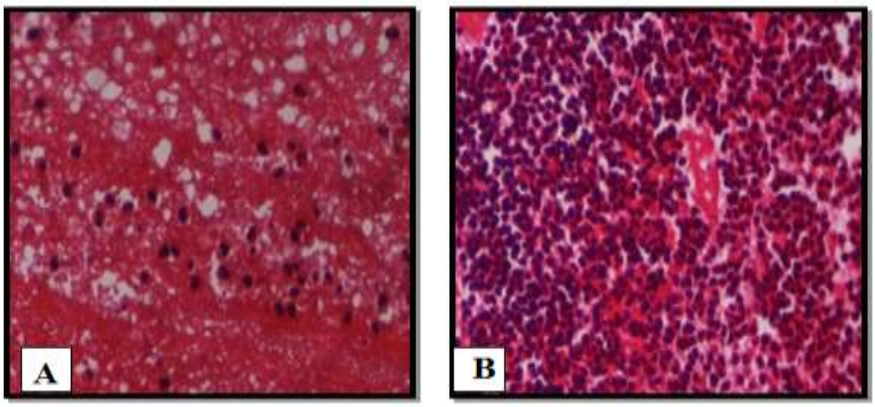


**Figure 3: Cell nests in the inner fibrin fibre zone in PRF membrane: (A) Score 1 = Inside, (B) Score 2= Outside.**

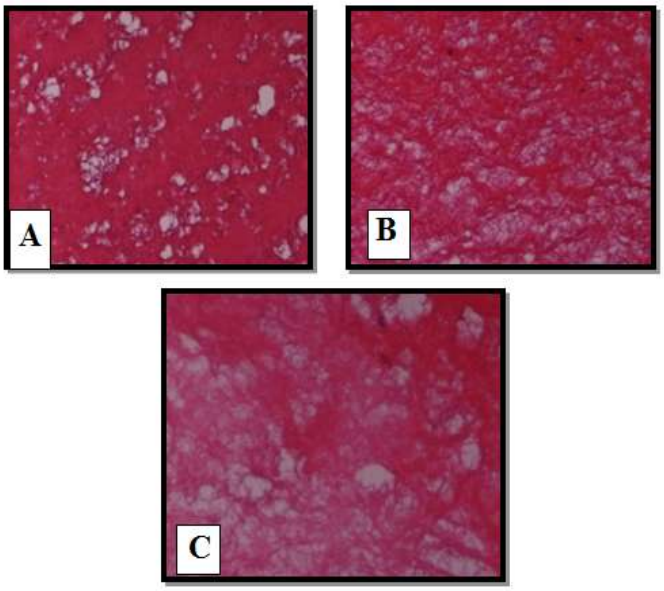


**Figure 4: Density of inner fibrin fiber zone in PRF membrane: (A) Score 1= Very dense, (B) Score 2= dense, (C) Score 3=Loose.**

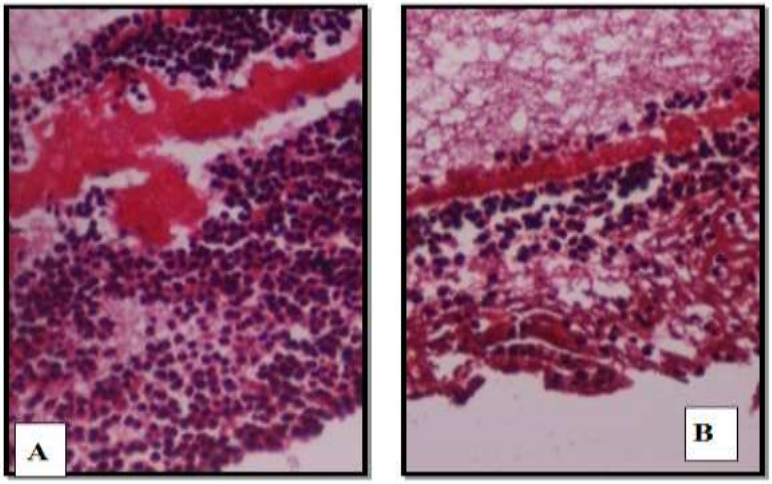


**Figure 5: Number of cell layers at border in PRF membrane: (A) Score 1= More than ten cell layers, (B) Score 2= Less than 10 cell layers.**

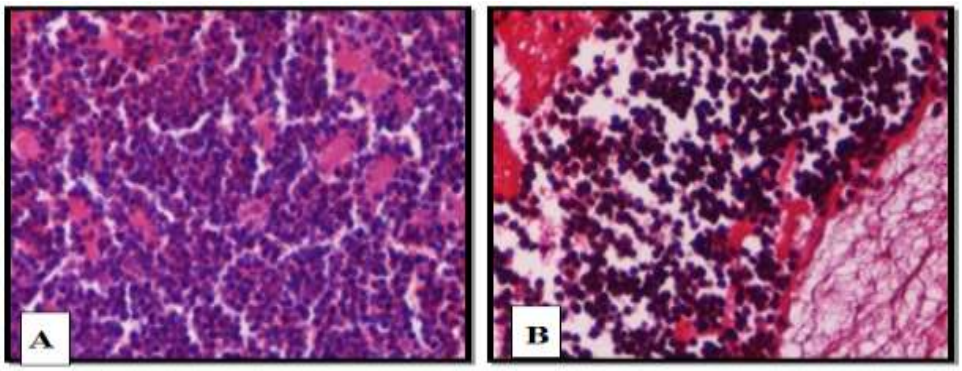


**Figure 6: Aggregation of cells in the cell layer zone PRF membrane: (A) Score 1= Heavy (B) Score 2= Light.**

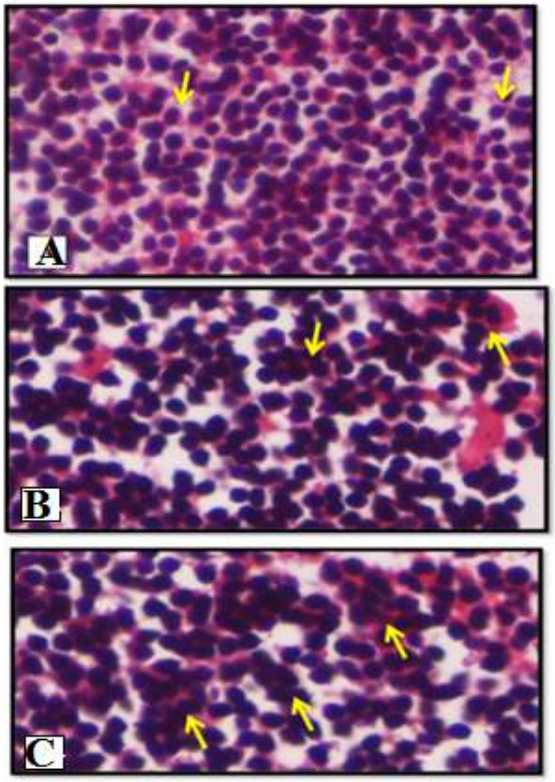


**Figure 7: Cell border morphology in PRF membrane: (A) Score 1= Very clear (B) Score 2= Unclear (C) Score 3= Very unclear.**

**Table -2: Histological criteria assessment for Platelet Rich Fibrin membranes (percentage and Chi-Square values) among three groups**

| | Zone layers in the membrane | | Cell nests in the inner fibrin zone | | Density of inner fibrin zone | | | Number of cell layers at border | | Aggregation of cells in the cell layer | | Cell border morphology | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model** | **Well dem.*** | **Poorly dem.*** | **Inside** | **Outside** | **Very dense** | **Dense** | **Loose** | **More than 10** | **Less than 10** | **Heavy** | **Light** | **Very clear** | **Unclear** | **Very unclear** |
| **Hettich** | 80% | 20% | 20% | 80% | 90% | 10% | | 90% | 10% | 90% | 10% | 20% | 70% | 10% |
| **Total** | 100% | | 100% | | 100% | | | 100% | | 100% | | 100% | | |
| **800-D** | 60% | 40% | 20% | 80% | 10% | 50% | 40% | 80% | 20% | 70% | 30% | 10% | 20% | 70% |
| **Total** | 100% | | 100% | | 100% | | | 100% | | 100% | | 100% | | |
| **80-2** | 50% | 50% | 10% | 90% | 20% | 50% | 30% | 80% | 20% | 80% | 20% | 10% | 10% | 80% |
| **Total** | 100% | | 100% | | 100% | | | 100% | | 100% | | 100% | | |
| **Chi-Square** | 2.01¶ | | 0.48¶ | | 16.12¶¶ | | | 0.48 ¶ | | 1.25 ¶ | | 12.07 ¶¶ | | |
| **Sig .** | 0.36 | | 0.78 | | 0.00 ** | | | 0.78 | | 0.53 | | 0.01** | | |

dem. = Demarcated , ¶ degree of freedom df = 2, ¶¶ degree of freedom df = 4 Sig. = Significance , ** Highly significant at $p \leq 0.01$

**Table 3: Mann-Whitney test comparison of two histology criteria and centrifuge used.**

| Centrifuge | Density of inner fibrin zone | | Cell border morphology | |
|---|---|---|---|---|
| | Value | Sig. | Value | Sig. |
| **Hettich vs. 800-D** | 8.00 | 0.00** | 21.50 | 0.02* |
| **Hettich vs. 80-2** | 13.50 | 0.00** | 17.50 | 0.01** |
| **800-D vs. 80-2** | 42.50 | 0.57 | 45.00 | 0.73 |

*Significant at $p \leq 0.05$ ** Highly significant at $p \leq 0.01$.

## DISCUSSION

In the current study, the original benchtop centrifuge device (Hettich) was used as the standard for comparison of its PRF clot with clots (membrane after histological processing) produced by the two other selected bench top centrifuge devices namely the model 800-D and model 80-2. The recommended relative centrifuge force or ***g***for the production of PRF clots is around 400. This light force imposed on the blood sample during the specific speed set or so called - soft spin allows the production of a good quality PRF clot in terms of platelet and leucocyte content enmeshed in a dense fibrin network , adequate size and biological nature (Dohan Ehrenfest, personal communication; 2014). In the current study, this ***g*** force was standarized for the models 800-D and model 80-2 centrifuge using the equation formula recommended [(28)].

The first centrifuge (Hettich) used in the current study (weight 4 kg / maximum speed 6000rpm ) was the original one during the early development of the PRF open – access method and is currently marketed under the name Intraspin™ PRF centrifuge ( Intra-lock International , Boca-Raton, FL,USA; made in Germany ) [(26)]. It is the only CE marked and FDA cleared system for the preparation of PRF clots with minimal heat generation, vibrations and noise level and was set as the standard model with its PRF product for comparison in the current study. The second centrifuge, model 800-D was a Chinese brand of low cost / small light weight (3.5kg) bench top centrifuge designed fo basic laboratory examinations with a maximum speed of 4000 rpm (enough for the production of PRF) and minimal to moderate vibration level. The third centrifuge, model 80-2 was also a Chinese brand of low cost / yet bigger and heavier than the other two centrifuges (7.5 kg) bench top centrifuge also designed for basic laboratory examinations with a maximum speed of 4000 rpm (enough for the production of PRF) and minimal to moderate vibration level.

The first histological description of a PRF membrane was conducted by Dohan Ehrenfest *et al.* [(29)] followed by Pinto *et al.*. [(30)]. The protocol for histological processing in their study for membranes were followed in the current study (except exact membrane thickness which was difficult to control). From there, a number of histological observations observed in the standard PRF membrane produced from the Hettich centrifuge were chosen and grouped together to form a new simple histological scoring design which aided in showing simularities and differences between different PRF membranes produced by other centrifuge models. As long as the histological processing is standarized, this design will assist greatly in defining PRF clots based on bright–light microscopy. In this context, the 800-D and 80-2 model membrane groups showed similarities to Hettich membranes being mostly described as membranes with a homogenous fibrin network with well demarcation of layers, heavy accumulation of cells at its periphary and few cell nests in the inner fibrin zone indicating a soft spin with acceptable separation of components in groups 800-D and group 80-2 likewise to Hettich group.Only two out of six observations showed significant difference among the three centrifuge groups, namely density of inner fibrin zone and clear cell wall morphology (to assess the viability of cells). As long as the processing protocol is respected, such differences between different membranes may be due to the effect of different heat generation levels on the blood sample during the spin as well as vibration levels in each centrifuge which define the specific fibrin architecture, cellular content and morphology of that PRF membrane produced [(26,31)] and (Dohan Ehrenfest, 2014; Personal communication). With respect to the original PRF membrane histological findings , the two membranes produced by the model 800-D model and 80-2 model in the current study did contain a heavy accumulation of leucocytes (netrophils and lymphocytes), thus defining both of them as PRF like products.

## CONCLUSION

Within the limitations of the current study, PRF clots can be produced by centrifuges that are cheaper and readily available yet at the same time should respect the original protocol and for that, such clots should be termed PRF-like products which is a term that could be added to the recent global classification.

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