International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 4 Issue 10, October -2015

Platelet-Rich Fibrin (PRF) Clot Temperature and Dimensions Produced by Three Centrifuges

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ABSTRACT

Aims: The aims of the current study were to show the differences between original Platelet-Rich Fibrin (PRF) clots produced by a specifically designed centrifuge for this purpose and those clots produced by other centrifuges in terms of clot temperature and clot dimensions.
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). The three produced clot were assessed and compared in terms of visual inspection and clot dimensions using a computer software program.
Results: For clot dimensions, a significant difference was observed between clots in terms of length but no significant difference in terms of clot width.
Conclusions: Differences in clot dimensions 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, Platelet concentrates.

I. INTRODUCTION

The first act of healing associates many actors and is one of the most complex mechanisms among the vital functions of living tissue [(1)]. 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 [(2, 3)]. A better understanding of wound healing and tissue regeneration processes has stimulated the research and development of new fields of research like regenerative medicine and tissue engineering to improve healing. The concept of "biological solutions to biological and medical problems" is rising as a new discipline in medicine leading to the development of optimal biological preparations that might open new opportunities in surgery for the treatment of a wide range of diseases, improve function and reduce costs. The use of blood derived products as surgical adjuvants to seal and stimulate wound healing is one of such trends [(4, 5, 6)].

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 [(10)]. 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 [(14-32)].

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 encouraged that changes in materials and /or protocols may affect considerably the PRF clot content and architecture and must therefore be categorized separately as a specific PRF-like product and not as the original PRF described in the literature. This argument 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 which may ultimately interfere with the quality and biological signature of the final PRF product [(33)], (Dohan Ehrenfest, personal communication, 2014).
The current conducted study was an attempt to compare three PRF clots 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 methodology for PRF clot sample temperature and dimension measurement is described.

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 and stethoscope (made in 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 vacutainer tubes (made in 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 according to a standard equation [34]. 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).

Temperature readings of PRF clot samples
At the completion of centrifuge cycle, the two collection tubes were immediately removed and placed into a plastic rack. The cover tops were removed and immediate temperature readings made by dipping the sensor lead probe of the digital multimeter (Thermocouple / Model DT-9208A® / China) into the serum fraction of the now produced PRF clot sample (Fig.1). After readings, the sensor lead probe was wiped with sterile dry gauze. A total of thirty readings (n=30) were taken.

Visual Inspection and Gross Measurement of PRF Clots
At completion of temperature reading, the clots were examined by the naked eye and then removed from the tube using tweezers and gently laid on a sterile damp (using normal saline to avoid sticking) gauze (Fig.2, A). Using the closed end of a scissor, the red blood cell base attached at its end was gently scraped off (Fig.2, B). Using tweezers and spatula from the kit, each clot was then gently placed on a glass microscopic slide (dimensions = 75mm * 25mm) (Fig.3) and (Fig.4). Using a digital camera (Sony Cyber-shot, 18,2 megapixel resolution/ Japan) fixed on a prefabricated camera stand and set to automatic mode capture with a fixed lens to glass slide distance of two centimeters (Fig.5), digital photo images of the clots were taken (total of ten clot images). The images were transferred to a computer and two dimension measurement (length and width set in millimeters) of each clot were measured using the line tool in image J free software program. The area selected for length measurement was the middle section of the clot body from superior to inferior as it was in the collection tube (Fig.6.A) and for width, a line intersecting the middle length distance of clot body from one side to the other (Fig.6. B). For each clot measured, two readings were taken for each dimension and means were considered.

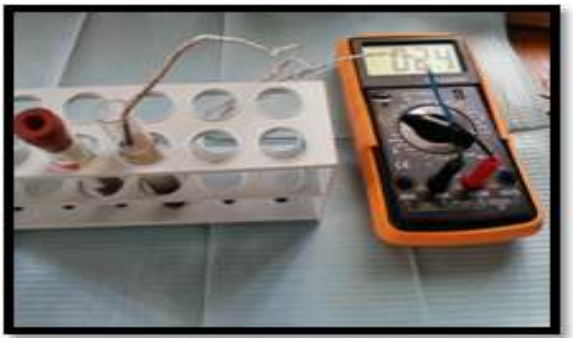

Figure 1: Temperature recordings of PRF clot samples

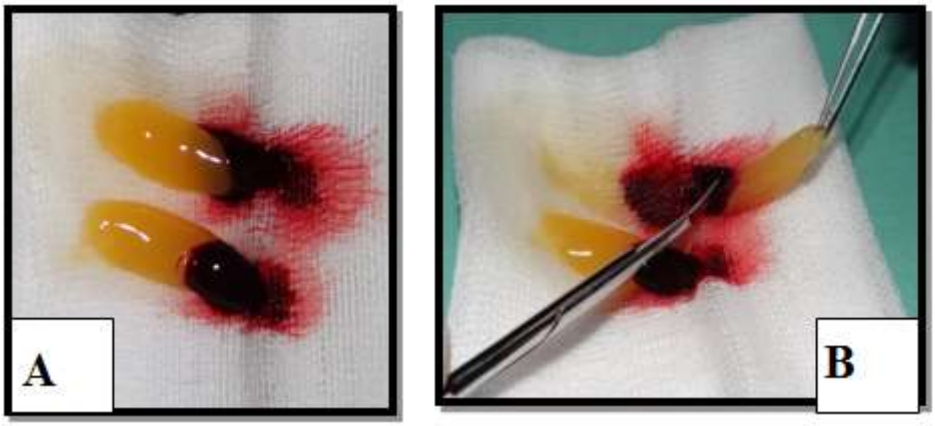

Figure 2: (A) Clots were laid on a sterile piece of gauze, (B) Using the closed end of scissor; the red blood cell base attached to the clot end was gently scraped off.

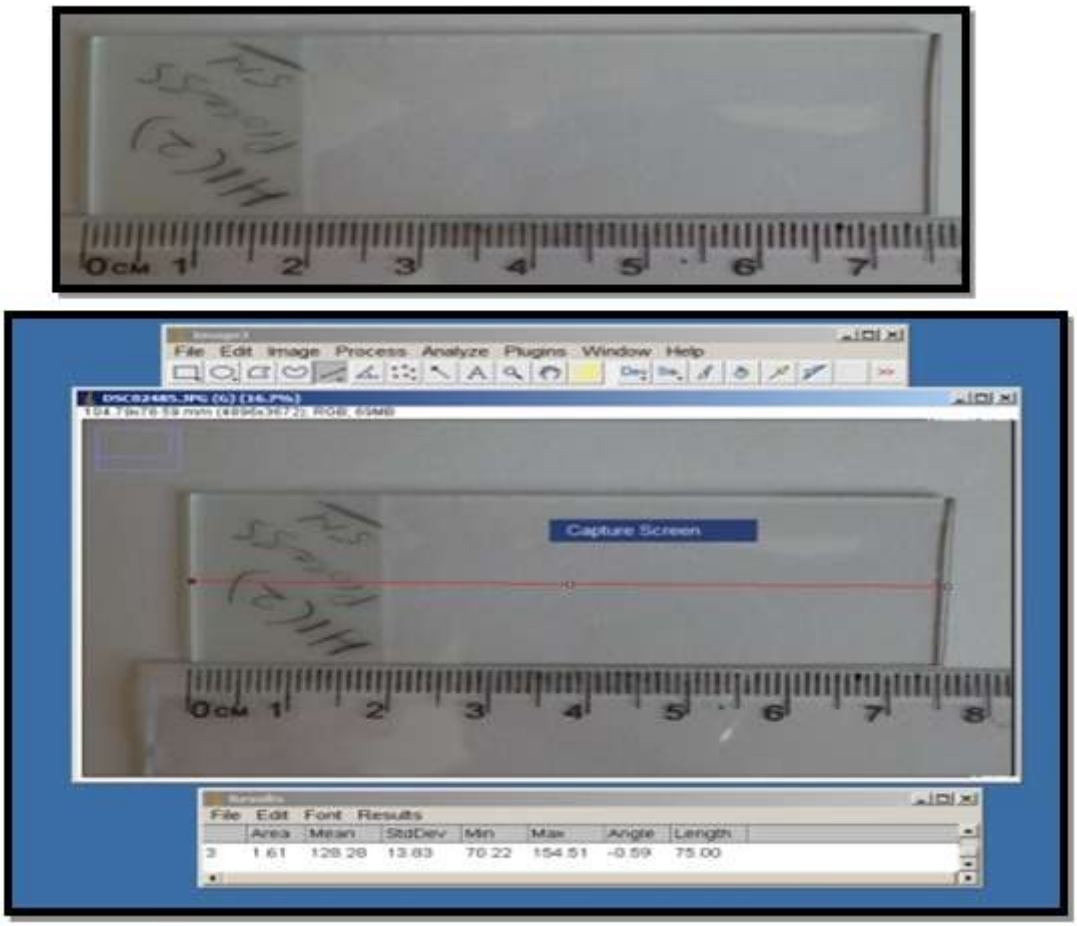

Figure 3: Glass microscopical slide dimensions = 70*25mm

Figure 4: Clot on slide

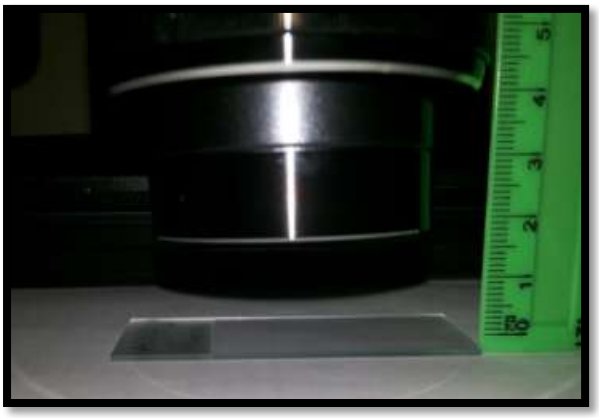

Figure 5: Standardization for digital photo- imaging of clot

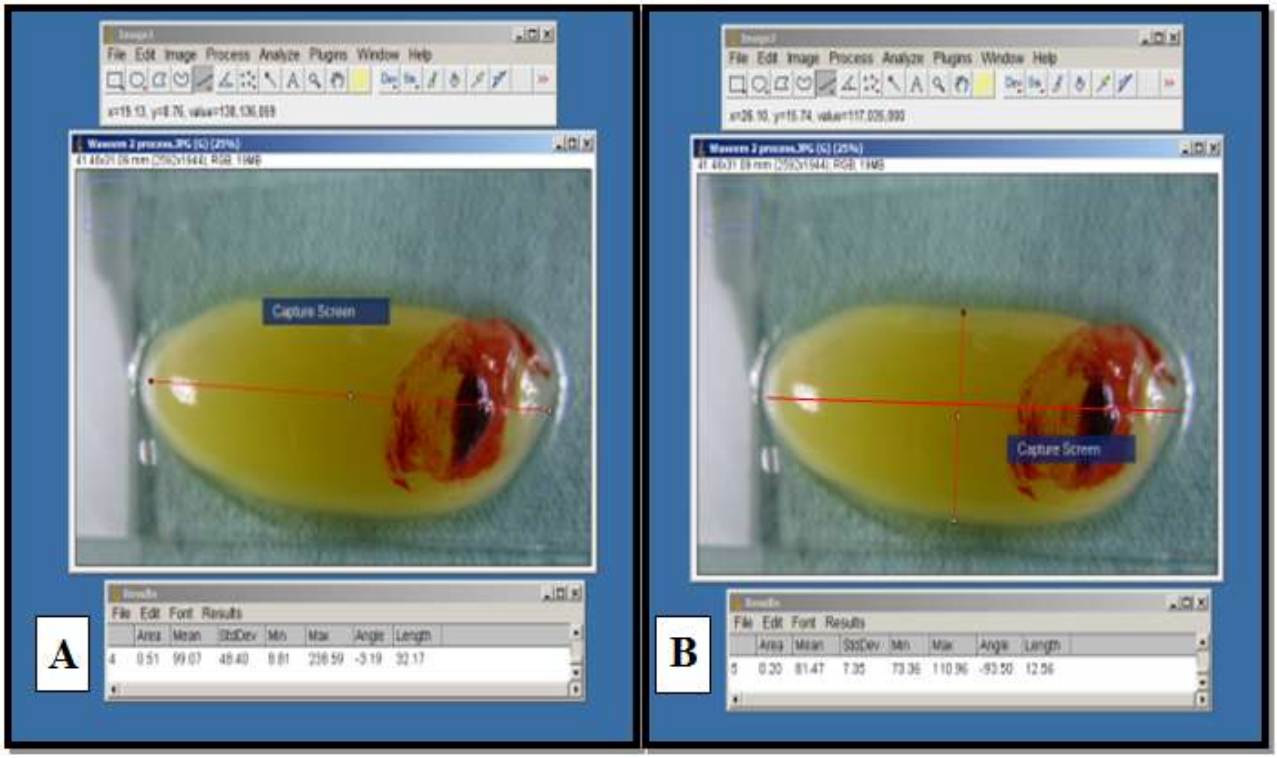

Figure 6: (A) Measurement of clot length, (B) Measurement of clot width using image j software program.

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: **Parametric data** included descriptive statistics and analysis of variance (ANOVA). Significant statistical difference was set at $\boldsymbol{p} \leq 0.05$ and $\boldsymbol{p} \leq 0.01$.

RESULTS

Temperature of Clot Samples

The mean temperature of the three clots produced immediately after the centrifuge spin were; in group Hettich was 26.20 C°, group 800-D was 30.6 C° and in group 80-2; 29 C°. In the Analysis of variance (ANOVA) and Post Hoc testing using Duncan, results showed a highly significant statistical difference between groups in regard to the least rise of centrifuge chamber temperature and in turn reflecting a reduction of clot sample temperature at the end of the centrifugation cycle with group Hettich having the highest reduction of clot sample temperature followed by group 80-2 and group 800-D (Table - 1).

Table–1: Mean clot sample temperature in volunteers.

Group	No.	•Mean	± SD	F value	Sig.	†Duncan
Hettich	10	26.20	0.42			A
800-D	10	30.6	0.17	37.2	0.00**	C
80-2	10	29.0	0.94			B

No. = Number, • Measurements in Celsius, SD= Standard deviation, ** Highly significant difference at $p \leq 0.01$, †Duncan grouping; different letters mean significant difference.

On direct visual examination of the collection tube, the clots were similar to each other as being described as composed of three main portions; from top to bottom; serum followed by a bulk fibrin portion and a red portion (Fig.1). Between the fibrin and RBC layer, a whitish colour was observed (buffy coat) assuming to contain WBC and platelets.

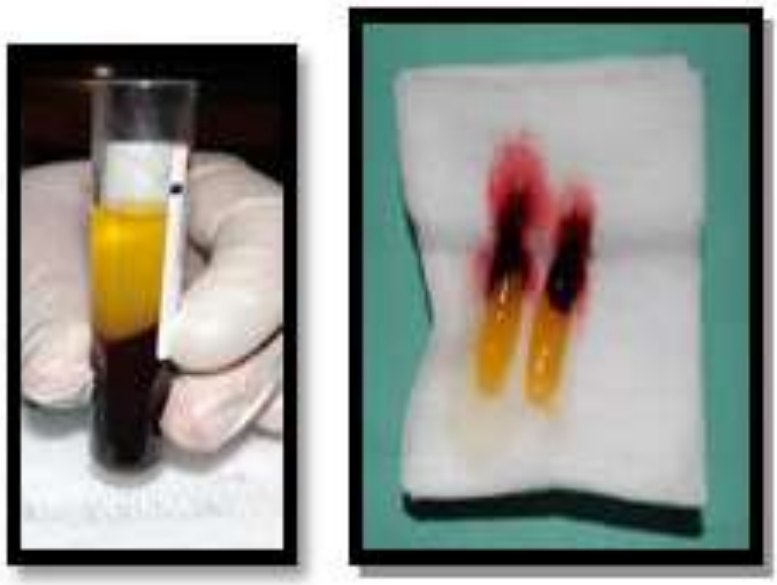

Figure 7: Direct visual inspection of PRF clots inside tube.

For human volunteers, descriptive analysis showed that the highest mean length was in group Hettich (35.30mm) followed by group 800-D (30.69mm) and group 80-2 centrifuges (27.15mm). For clot sample width, the highest mean was in group Hettich (13.32mm) followed by group 800-D (12.49mm) and group 80-2 centrifuge (12.52mm) as in (Table -2)**.**

Table -2: Descriptive analysis of clot dimensions of volunteers

Group	Length*				Width*			
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD
Hettich	32.55	38.82	35.30	2.06	11.80	14.95	13.32	0.78
800-D	26.74	34.24	30.59	3.45	10.82	13.54	12.47	0.69
80-2	20.45	33.44	27.15	3.67	10.48	13.82	12.52	1.11

Min. = Minimum, Max. =Maximum, SD= Standard deviation. * Measurement in millimetres.

In the Analysis of variance (ANOVA) and Post Hoc testing using Duncan, results showed a highly significant difference between groups in clot sample length (longest in group Hettich followed by group 800-D and group 80-2), but showed no statistical difference regarding clot width between groups (Table -3).

Table 3: Comparison of human clot dimensions among three centrifuges

Variable	Group	No.	Mean•	± SD	F	Sig.	Duncan†
Length	Hettich	10	35.30	2.06	16.86	0.00**	C
	800-D	10	30.59	3.45			B
	80-2	10	27.15	3.67			A
Width	Hettich	10	13.32	0.78	2.92	0.07	A
	800-D	10	12.47	0.69			A
	80-2	10	12.52	1.11			A

No. = Number, • = Measurements in millimetre, SD= Standard deviation, ** Highly significant difference at $p \leq 0.01$, †Duncan grouping; different letters mean significant difference.

DISCUSSION

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), [33]. 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 for 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 differences in heat generation between different centrifuges may result from differences in friction of the centrifuge rotor during the spin, size of the drive itself, number of vents in the centrifuge body to allow heat generated to escape and whether or not there is a built-in cooling system (Ford and Graham, 1991).

All three devices were brand new on commencing the study, and on inspection from the inside, the drive of the model Hettich was seen to be the smallest followed by the drive of the model 800-D and biggest in the model 80-2. In addition, the drive of the model Hettich centrifuge was completely isolated from the above tube containing rotor by a thick plastic shield, a feature not seen on inspection of both models 800-D and model 80-2 centrifuges. All three centrifuge devices did not have a built-in cooling system. In regard to vents in the body of centrifuge, the Hettich centrifuge had 20 air vents at it backside, while for the models 800-D and Model 80-2 centrifuges, ten vents for each centrifuge were seen at it backside. This could also explain the rise of temperature inside the chambers of the model 800-D and model 80-2 where heat escape was hindered. None of the three centrifuges used in this study had a built-in cooling system. In the PRF clots produced and for three centrifuge groups, visual inspection of clots came in agreement with previous studies with a standard bulky PRF clot being comprised of three portions (serum fraction, bulky yellow colored fibrin portion and a bottom red blood cell layer [9,13,35]. In regard to clot dimensions in the current study, a highly significant difference was disclosed in terms of clot length with group Hettich being longest but no significant difference in terms of width (35.30mm * 13.32mm) , group 800-D (30.59mm * 12.47) and group 80-2 (27.15 * 12.52). Pinto *et al.*, compared PRF clots of human volunteers in their four different centrifuge comparison study and showed that clots produced from the Hettich centrifuge and Salvin group centrifuge were higher in terms of length and width (35.69mm; 12.81mm),(35.25mm; 10.93mm),respectively when compared with two other centrifuges (A-PRF) and LW centrifuge models (26.56mm * 10.93mm), (20.12 * 9.12mm) respectively. The dimensions of PRF clots from the Hettich centrifuge in the current study were very close to those produced by the study mentioned above. At the same time, the dimensions of group 800-D clots and group 80-2 clots were higher than the PRF clots produced by the brands (A-PRF) and (LW) centrifuge groups in that study. The explanation for differences in the dimensions of clots would seem to be related to each centrifuge , namely its mechanical specifications since blood samples from each volunteer were equally allocated to the three centrifuges (total of six samples / two allocated for each centrifuge) thus individual variation was avoided. The differences in dimensions between the human clots produced by different centrifuges in the current study may be related to differences in heat generated inside its chamber and level of vibrations of the centrifuge itself during the spin. This may be explained by that different vibrations during the centrifugation process in different centrifuges provoke the phenomenon of resonance, with cavitation's formed within the blood sample tubes surplused by heat surroundings greatly affecting the quality of fibrin polymerisation, thus resulting in different dimensions [33] , (Dohan Ehrenfest, personal communication, 2014).

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 a PRF-like product which is a term that could be added to the recent global classification.

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