Letter to the Editor regarding Fujioka-Kobayashi et al. 2017 (JOP-16-0443.R1)

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Dear Editor, please find enclosed a “letter to the editor” which I hope you could consider for publication, because it is extremely important to understand data from several studies, and is crucial to consider in the future.

‘for figures, tables and references, we refer the reader to the original paper’

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Letter to the editor:

Leukocyte- and platelet-rich fibrin (L-PRF), a 2nd generation platelet concentrate, has become of great interest in periodontology (e.g. Castro et al. 2016, 2017), and extra-oral wound healing (e.g. Pinto et al. 2017). The preparation of L-PRF is based on a centrifugation process, aiming for a blood clot containing the majority of leucocytes and platelets, without the inclusion of red blood cells (Dohan et al. 2010).

The final composition (including the release of growth factors, physical characteristics, ...) as well as the clinical performance of such platelet concentrate depends on several aspects (Figure 1), including the time of spinning, the speed (rotation/revolutions per minute, RPM) and the g force (relative centrifugal force, RCF), but also differs significantly between centrifuges (Dohan et al. 2017). The latter is explained by variations in: rotor diameter, rotor angulation (Table 1), and rotor radial vibration (Dohan et al. 2017).

When evaluating and definitively when comparing medical devices and protocols in this arena, factual accuracy is of utmost importance. Even a centrifugation at the same RPM will exert different centrifugal forces if the centrifuge rotors have different radius sizes, bucket types or bucket sizes (Table 1, Pendleton 2016).

We therefore, would like to bring to your attention what we believe to be misinformation in a recent article entitled: “*Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response*” published in the Journal of Periodontology (2017). This paper compares 2 protocols for producing a leukocyte-and platelet-rich fibrin clot:

- the standard “L-PRF” (standing for “leucocyte and platelet rich fibrin”),
- and the “A-PRF” (standing for “advanced PRF”).

In the materials and methods section of the above-mentioned article, L-PRF is processed at 708 RCF (2700 RPM in Duo Process centrifuge, Table 1, Figure 2), which is far above the standard protocol of the original L-PRF (± 408 RCF, Dohan et al. 2006, Choukroun et al. 2006). This higher g-force unfortunately has a number of negative side effects with significant impact on the outcome of the study:

- a higher g force will push more cells to the bottom of the tube (Figure 2) and as such reduces the cellular content in the clot (de Melo et al. 2018),
- at higher g force the PRF clot becomes poorer in platelets and leucocytes, and as such will release less growth factors (Kobayashi et al. 2016, Ghanaati et al. 2014),
- at higher g force, the radial vibration increases exponentially, which has a negative impact on the clot formation and its fibrin network (Dohan et al. 2017), especially in a device that has already a high vibration by itself (Dohan et al. 2017), and will definitely lead to cell damage, again leading to a reduced release of growth factors.

These aspects might explain why A-PRF (in this paper prepared at 1300 RPM, Table 1) showed an increase in growth factor release, and superior fibroblast migration, proliferation, and collagen mRNA levels in comparison to a misrepresented specimen of L-PRF (Figure 2).

In case one would like to compare two different platelet concentrates it is crucial to follow the original protocols for each product (specific centrifuge, specific setting and specific blood tubes). When a different centrifuge is used in the production, the speed (RPM) during the centrifugation has to be adjusted to the characteristics of the new centrifuge (radius and angulation of the rotor, Table 1), in order to generate the intended relative g force (RCF). Even then, a different product will be obtained (Figure 2). The best way is of course to compare with the original centrifuge and the original setting.

In order to measure the release of growth factors from the respective fibrin clots, the authors mentioned using the top 4 ml from the tubes. This again is misleading, since a centrifugation at 708 RCF for 12 minutes will push the red blood cells but also the platelets and leucocytes further to the bottom (Figure 2) of the tube. As such the top 4 ml in the tube will miss a large number of the platelets and leucocytes. The latter again explains why the authors noticed less cell activity in the L-PRF clots. In order to assess the growth factors activities from the fibrin clot in any of the compounds, it would make more sense to take the “complete fibrin clot” from the tube, and gently peel away the remaining red cells. This way all the cells in the fibrin clot are selected and are allowed to contribute.

These shortcomings in the centrifugation protocol in the study of Fujioka-Kobayashi and co-workers (2016) might explain why Dohan and co-workers (2017) in fact reported the opposite when comparing L-PRF with A-PRF, with in their paper the latter having less cell activity.

Therefore, the entire premise of this paper is highly questionable; in fact the standard L-PRF control was negatively influenced. We firmly believe this important information should be noted and if possible corrected in the above-mentioned Journal of Periodontology article. Thank you in advance for your consideration.