Selecting a relevant in vitro cell model for testing and comparing the effects of a Choukroun's platelet-rich fibrin (PRF) membrane and a platelet-rich plasma (PRP) gel: Tricks and traps

To the Editor:

We recently read with a great interest an article published in *OOOOE*, where He et al.[1] tried to compare the effects in vitro on the proliferation and differentiation of rat osteoblasts induced by a platelet-rich plasma (PR) and a platelet-rich fibrin (PRF). We have a significant experience with cell cultures using PRF,[2,3] and the method and terminology used by He et al. raise several questions that require discussion.

In their study, He et al. decided to incubate PRF and PRP in culture conditions for 28 days and to collect their exudates at 5 experimental times: 1, 7, 14, 21, and 28 days. The authors then tested the effects of these exudates on cell cultures. The advantage of this approach is to limit interspecies immune reactions between human PRP/PRF and rat cell cultures. The problem is that the authors tested the effects only of the growth factors and proteins in suspension in the PRP/PRF exudate and not the true effects of the whole fibrin product. This is a significant limitation, because PRF is a dense fibrin-based solid biomaterial[4] with a specific matrix architecture and cell content (platelet aggregates, activated leukocytes),[5] and its exudate is clearly not the most interesting part of the product. Similarly, even if most PRPs are homogeneous liquid platelet suspensions, their exudates are not the sole bioactive components, because platelet aggregates themselves and their associated fibrin gel also trigger many biological mechanisms.[6,7]

Regarding the PRP, the authors claimed to use the Curasan method (Kleinostheim, Germany), but unfortunately they did not evaluate the leukocyte content of their final product.[8-10] Curasan PRP is indeed classified in the leukocyte- and platelet-rich plasma (L-PRP) family.[4] Leukocytes are turntables of the growth factor release and play a significant role in the biology of these products.[11-14] Moreover, the authors did not point out how and when they activated their PRP (using thrombin or batroxobin), and it seems that the PRP was in fact never activated into a gel. This approach is debatable, because the true final product used in patients is an activated L-PRP gel (in most cases), not an unactivated platelet suspension.[15,16]

Regarding the PRF, there are very serious questions about the protocol described by He et al. Indeed, the authors claimed to use the "PCCS protocol" (Platelet Concentrate Collection System; 3I, Palm Beach Gardens, FL, USA) to produce PRF, and referred to our own references about the PRF technology.[17,18] The problem is that Choukroun's PRF can not be produced with the PCCS device, because that centrifuge and tubes are specifically designed for the automated production of an L-PRP.[8,9] Moreover, the authors claimed to have quantified the platelets in PRF, but that is impossible. Indeed, the production concept of PRF is based on the activation of platelets during the centrifugation to constitute strong fibrin/platelets strands and a dense final PRF clot[19]; therefore, PRF exists only in a solid material form, never in a liquid form. An accurate and direct quantification of the platelet content is impossible, and we showed only recently and indirectly by soustraction that >95% of the platelets from the initial blood harvest were merged in the PRF clot.[5] Consequently, we have no clear idea about the "PRF" the authors claimed to use in their study.

PRF is a free and open-access technique.[20] However, the name PRF is a trademark and refers to a specific preparation kit (PC02 centrifuge and collection kits; Process, Nice, France), production process, and final product. This trademark protection was not performed for industrial reasons, but to identify clearly the technique and protect it from confusion or scientific misunderstandings. Because there is no significant commercial interest in selling PRF devices, the only way to protect PRF was to force people to respect the definition of the product and thus to respect the trademark. In this article, it seems that the tested product was a liquid platelet concentrate produced with the PCCS, and maybe not an original Choukroun's PRF. Care should be taken to respect a clear and reliable terminology[4] to avoid significant misunderstandings in the literature.

Finally, testing the in vitro effects of complex living biomaterial such as Choukroun's PRF clot or membrane requires the greatest caution to get reliable results. Whatever the method, it is important to test the whole PRF and not just fragments,[2] because PRF presents a specific 3-dimensional architecture and cell distribution.[5] Moreover, care must be taken to always keep PRF in an osmotic medium (such as culture me-

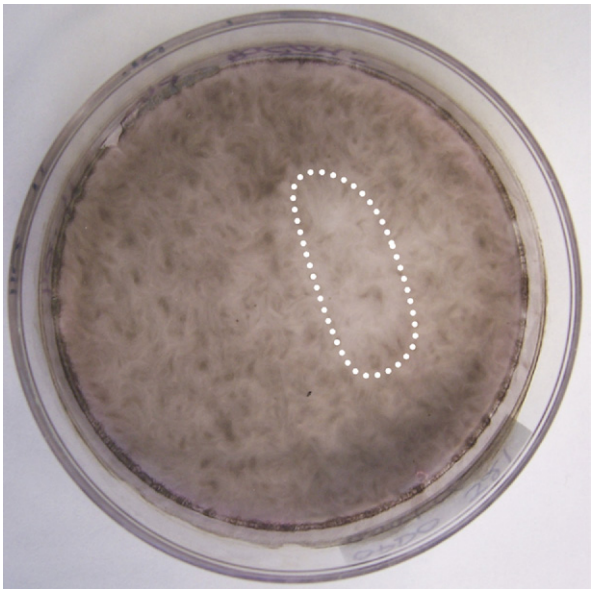

Fig. 1. Human osteoblast primary culture in contact with a platelet-rich fibrin (PRF) membrane in a large culture plate (60 mm diameter) after 21 days of culture. The plate was treated with Von Kossa staining showing mineralization nodules in black. The PRF membrane was removed after 7 days, but its trace is still visible in the middle of the plate (*dotted outline*).

dium) to preserve the cell content (particularly leukocytes) from deterioration.[21]

In their study, He et al. used an interesting indirect method for testing the effect of the exudates, but we think that they did not truly test the complete material as used in patients. It is therefore impossible to draw extensive conclusions with this specific methodology. From our own experience,[2,3] a complete and reliable in vitro methodology for testing the cell effects of a PRP or a PRF requires:

To use human cells in primary cultures from the same donor as for the platelet concentrate, to avoid immune cross-reactions and other related bias. Because it is impossible to produce PRF in rats, rat cells should be avoided.

To introduce the whole activated product, such as an activated PRP gel or the whole PRF clot or membrane, into the culture plate, to observe the complex reactions and equilibrium between the cultivated cells and the biomaterial. As shown in Fig. 1, the culture plate should always be large enough to receive a complete clot (and even several clots when testing a dose-dependent effect) without squeezing the cultivated cells. In our studies with Choukroun's PRF,[2,3] we always used culture plates with a minimum diameter of 60 mm, giving space for a full interaction between cells and biomaterial and allowing the expression of various patterns of cell development.

To consider the key role of leukocytes, when present.[14,22] Indeed, Curasan PRP and Choukroun's PRF are respectively an L-PRP and an L-PRF[4] and contain leukocytes. Any cell cultures with products from these families are necessarily cocultures with leukocytes, as was recently published.[2,3]

The analysis of the results obtained with PRP/PRF is thus particularly complex, but also truly exciting, because the biologic interactions and mechanisms of these blood products have yet to be identified and elucidated. Cell models with PRF are of particularly great interest, because Choukroun's PRF is an optimized blood clot[5]: all cell models with PRF are also investigations about the healing process after coagulation, with a potential interest beyond the strict field of the platelet concentrates for surgical use. This field of research, however, requires following a strict terminology and methodology to avoid the many scientific traps and associated misunderstandings.

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doi:10.1016/j.tripleo.2010.05.056

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In reply:

We have carefully read the letter by David M. Dohan Ehrenfest et al. regarding our article "A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro."

We respect Dr. Dohan Ehrenfest's pioneer work in PRF research. The purpose of our article was to evaluate the releasing pattern of growth factors in PRP and PRF and their effects on osteoblastic proliferation and differentiation. Our work was based on Dr. Dohan Ehrenfest's study that growth factors in PRF could release progressively and that this releasing pattern is thought to be one of the major differences between PRP and PRF in biologic activity.[1] We would like to provide our detailed study materials and reply to Dr. Dohan Ehrenfest's questions as follows.

WHY EXUDATES, NOT WHOLE PRF?

In 2007, when we tried to study the releasing pattern of PRF, we did not read any study about growth factor–releasing patterns of PRF with exudate models, nor reports on its cell effects; therefore, we could not follow any reported protocol from the previous literature to design our work. In fact, our study showed the different releasing patterns of platelet-derived growth factor (PDGF) AB and transforming growth factor (TGF) β1 in exudates of PRF and PRP. Furthermore, we determined the different effects of exudates of PRP and PRF on osteoblastic proliferation and differentiation. The results of our study showed that exudates of