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Letter to the Editor

Why dilute the regenerative power of platelet-rich plasma?

To the Editor:

We have read with great interest the article by Vukelic-Nikolic et al. entitled "Osteogenic capacity of diluted platelet-rich plasma (PRP) in ectopic bone-forming model: Benefits for bone regeneration" (Vukelic-Nikolic et al., 2018). In vivo osteogenic capacity of bone mineral matrix (BMM), alone or in combination with two different PRP diluted samples, PRP diluted 3 times (dPRP/3) and 10 times (dPRP/10), was evaluated 1, 2, 4 and 8 weeks after implantation. The authors demonstrated that all treatment groups included in this study show similar osteogenic potential along the study. They concluded that diluted PRP could be used as an adjuvant therapy in bone regeneration, and that it should not be necessary to obtain a concentrated PRP to achieve a successful result in the clinical application.

We congratulate the authors for this new publication; at the same time, we would like to make some comments and stimulate discussion on the use of PRP therapies.

First of all, with regard to the methods, it is not specified whether the blood is obtained from a single mouse or from several animals and then the PRP pooled. On the other hand, the authors state that they obtained a platelet concentration of $9.9 \times 10^5/\mu L$ in the PRP, while referencing an average peripheral blood value of $8.9 \times 10^5/\mu L$, so theoretically they obtained an increase of only 1.1-fold with respect to peripheral blood. In addition, the obtaining method that is referenced in the paper (Vukelic-Nikolic et al., 2018) obtains "a platelet increase of 8–10 fold above the physiologic levels" (Intini et al., 2007). We believe that it is necessary to report adequately the methods for obtaining PRP and characterize its composition, since variations in the concentration of platelets and leukocytes can produce different clinical outcomes. Furthermore, in this way, it would be possible to reproduce and compare the results obtained in different studies.

On the other hand, the authors state that the platelet concentration of $8.9 \times 10^5/\mu L$ obtained in PRP samples in the study corresponds to the physiological level of platelet in mouse peripheral blood. Hence, blood-derived samples obtained in the present study should not be considered as plasma enriched in platelets.

Second, several *in vitro* studies have shown that there was a dose-response effect when osteoblasts (Anitua et al., 2013) and other cell types (Anitua et al., 2009) were exposed to different PRP concentrations. The effect disappeared when PRP reached a certain platelet level threshold, but these concentrations are higher than the authors' assay in their research. Furthermore, several clinical studies demonstrated that concentrated PRP improve bone regeneration in comparison with both bone mineral matrix alone (Anitua, 1999; Torres et al., 2009) or blood clot (Anitua et al., 2015). In addition, although authors discuss that similar histological findings were observed in other studies using PRPs enriched in platelets (Cvetkovic et al., 2015; Najdanovic et al., 2015; Najman et al., 2016; Zivkovic et al., 2015), most of them used diluted PRP or blood to obtain the different samples to be evaluated (Cvetkovic et al., 2015; Najdanovic et al., 2015; Zivkovic et al., 2015). Only Najman et al. used concentrated PRP in their study showing significant differences in osteogenic capabilities in the PRP group in comparison to the control group (Najman et al., 2016).

Third, another issue of concern is whether the PRP, diluted 1/10, or even 1/3, allows an adequate embedding of bone mineral matrix, since in clinical practice is necessary to have a biomaterial with enough consistency. The PRP agglutination capacity would be diminished when it is diluted. An additional advantage of the formation of a combined scaffold of PRP and the bone mineral matrix is that it improves both handling and administration in the lesion while avoiding the uncontrolled displacement of Bio-Oss particles (Anitua et al., 2012).

Finally, we do not agree with the conclusions by the authors related to their affirmation that it should not be necessary to obtain concentrated PRP to achieve a successful result in the clinical application. In this sense, we encourage the authors to carry out a comparative research between diluted PRP and conventional (undiluted) PRP, in order to evaluate the osteogenic potential of both treatments.

Conflicts of interest

The authors declare the following conflicts of interest: EA is the Scientific Director of and RP and FM are scientists at BTI Biotechnology Institute, a dental implant company that investigates in the fields of oral implantology and PRGF-Endoret technology.

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Eduardo Anitua*, Roberto Prado, Francisco Muruzabal
Eduardo Anitua Foundation for Biomedical Research, Vitoria, Spain

BTI-Biotechnology Institute, Vitoria, Spain

University Institute for Regenerative Medicine & Oral Implantology – UIRMI (UPV/EHU-Fundación Eduardo Anitua), Vitoria, Spain

* Corresponding author. Eduardo Anitua Foundation, Jacinto Quincoces, 39, 01007 Vitoria, Spain.
E-mail address: eduardo@fundacioneduardoanitua.org (E. Anitua).

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