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PRF TECHNIQUE AND ASSOCIATED GROWTH FACTORS

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Abstract

Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates geared to simplified preparation without biochemical blood handling. In this initial article, we describe the conceptual and technical evolution from fibrin glues to platelet concentrates. The slow polymerization during PRF preparation seems to generate a fibrin network very similar to the natural one. Such a network leads to a more efficient cell migration and proliferation and thus cicatrisation DuringPRF processing by centrifugation, platelets are activated and their massive degranulation implies a very significant cytokine release. PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes. This result would imply that PRF, unlike the other platelet concentrates, would be able to progressively release cytokines during fibrin matrix remodeling; such a mechanism might explain the clinically observed healing properties of PRF.

PLATELET-RICH FIBRIN—A NATURAL FIBRIN MATRIX

Technique

PRF was first developed in France by Choukrounet al. 70 for specific use in oral and maxillofacial surgery. This technique requires neither anticoagulant nor bovine thrombin (nor any other gelling agent). It is nothing more than centrifuged blood without any addition, which makes it possible to avoid all the restrictions of the French law related to blood-derived product reimplantation. This technology requires a PC-02 table centrifuge and a collection kit from Process (Nice, France).

The PRF protocol is very simple:

A blood sample is taken without anticoagulant in 10-mL tubes which are immediately centrifuged at 3000 rpm (approximately400g according to our calculations) for 10 minutes. The absence of anticoagulant implies the activation in a few minutes of most platelets of the blood sample in contact with the tube walls and the release of the coagulation cascades. Fibrinogen is initially concentrated in the high part of the tube, before the circulating thrombin transsforms it into fibrin. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top.

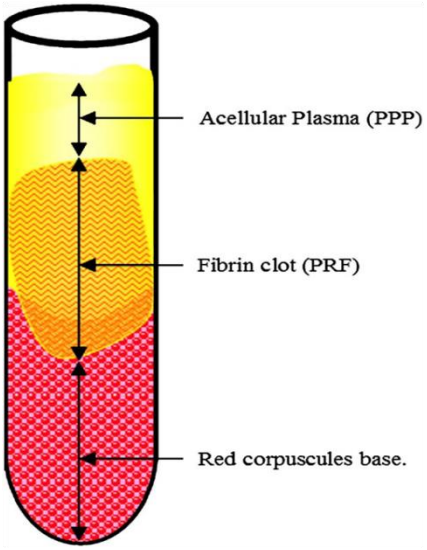

Fig. 1. Blood centrifugation immediately after collection allows the composition of a structured and resistant fibrin clot in the middle of the tube, just between the red corpuscle sat the bottom and acellular plasma at the top.

Platelets are theoretically trapped massively in the fibrinmeshes. The success of this technique entirely depends on the speed of blood collection and transfer to the centrifuge. Indeed, without anticoagulant, the blood samples start to coagulate almost immediately upon contact with the tube glass, and it takes a minimum of a few minutes of centrifugation to concentrate fibrinogen in the middle and upper part of the tube. Quick handling is the only way to obtain a clinically usable PRF clot. If the duration required to collect blood and launch centrifugations overly long, failure will occur: The fibrin will polymerize in a diffuse way in the tube and only a small blood clot without consistency will be obtained.

In conclusion, the PRF protocol makes it possible to collect a fibrin clot charged with serum and platelets. By driving out the fluids trapped in the fibrin matrix, practitioners will obtain very resistant autologous fibrin membranes.

PLATELETS, HEMOSTASIS, AND CICATRIZATION

Biologic mechanisms

Although platelet rich fibrin (PRF) looks like an autologous fibrin gel with cicatricial properties, it is actually anew platelet concentrate concept.[1-4] Its production protocol attempts to accumulate platelets and the released cytokines in a fibrin clot.

Formed in bone marrow from megacaryocytes, plateletsare discoidal and anuclear structures. Their lifespan is 8 to10 days, and the cytoplasm contains many granules whose contents are secreted at the time of activation. a-Granules contain many proteins, platelet specific(such as b-thromboglobulin) or non platelet specific(fibronectin, thrombospondin, fibrinogen, and other factors of coagulation, growth promoters, fibrinolysis inhibitors, immunoglobulins, etc.). The dense granules contain calcium, serotonin, etc. Moreover, the platelet membrane is a phospholipid double layer into which receptors for many molecules are inserted (collagen,thrombin, etc.).

Activation is fundamental to initiate and supporthemostasis because of aggregation on the injured siteand interactions with coagulation mechanisms. However,degranulation also implies the release of cytokines ableto stimulate cell migration and proliferation within thefibrin matrix, launching the first stages of healing

Platelet cytokines

TGFb-1: Fibrosis agent. Transforming growth factor b (TGFb) is a vast super family of more than 30 members. The reference molecule in speaking about ''the''TGFb will actually be TGFb-1. It is the most massively produced isoform, not only in the platelet a-granules, but also in general during intercellular dialog.[9] In vitro, its effects are extremely varied according to the amount applied, the matrix environment and cell type. For example, it has been shown that it could stimulate the proliferation of osteoblasts just as easily as it could cause their inhibition.[10] Although its effects in terms of proliferation are highly variable, for the great majority of cell types, it constitutes the most powerful fibrosis agent among allcytokines.11 In other words, it will induce a massive synthesis of matrix molecules such as collagen I and fibronectin, whether by osteoblasts or fibroblasts

Thus, although its regulation mechanisms are particularly complex, TGFb-1 can be considered as an inflammation regulator through its capacity to induce fibrous cicatrization.

PDGFs: Stimulant of mesenchymatous lineages. **PDGFs** (platelet-derived growth factors) are essential regulators for the migration, proliferation, and survival of mesenchymatous cell lineages.[12,13] According to the distribution of their specific receptors, they are able to induce stimulationas easily as inhibition of the development of these

cells.14 This position of regulation node plays a fundamental role during the embryonic development and all tissue remodeling mechanisms. For this reason, PDGFs play a critical role in the mechanisms of physiologic cicatrisation and the pathogenesis of atherosclerosis and many other fibro proliferative diseases (eg, neoplasia and pulmonary

and renal fibrosis).[15]

The IGF axis: Cell-protective agent. Insulin-like growth factors (IGFs) I and II are positive regulators of proliferation and differentiation for most cell types, which unfortunately include tumor cells (which use the IGF system to increase their survival potential).[16] Although these cytokines are cell multiplication mediators, in the main they constitute the major axis of programmed cell death(apoptosis) regulation, by inducing survival signals protecting cells from many matricial apoptotic stimuli.[17] Moreover, even though IGFs are released during platelet degranulation, they are initially massively present in blood circulation.

PLATELETS AND PRF

Platelet distribution in PRF

Preliminary hematologic studies revealed that platelet in the acellular supernatant (platelet-poor plasma (PPP)) or in the red blood corpuscles base, did not remain.

A few histologic analyses were sufficient enough to determine the platelet distribution within the various layers of the centrifuged collection tube: They accumulate in the lower part of the fibrin clot, mainly at the junction between the red corpuscles (red thrombus) and the PRF clot itself (Fig. 1). This last observation under scores the idea that the PRF red extremity would be of interest for clinical use and even more effective than the higher part of the fibrin clot (Fig. 2). Lastly, it is of high interest to note that the PRF matrix enmeshes glycosaminoglycans (heparin, hyaluronic acid) from blood and platelets. Their histologic aspect after alcianblue staining (Fig. 3) follows the fibrillary architecture of fibrin, meaning that these glycanic links are incorporated within fibrin polymers. Glycosaminoglycans have a strong affinity with small circulating peptides (such asplatelet cytokines) and a great capacity to support cell migrations and healing processes.[18]

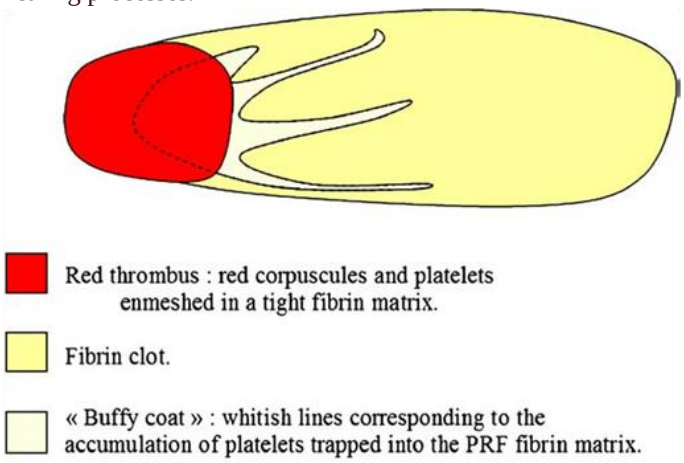

Fig. 2. The PRF fibrin clot obtained according to the Process protocol is divided into 3 parts: a red thrombus in contact with the red blood corpuscle base, an acellular fibrin gel, and a network of buffy columns corresponding to platelet accumulation.

Indeed, during PRF processing, the absence of anti coagulant in the collection tube necessarily induced massive platelet activation, bolstered by the presence of a mineral phase on the tube walls (residual glass particles). These cytokines are small soluble molecules which centrifugation could naturally concentrate in the upper part of the tube, in other words, in the supernatant.

However, this is clearly not the case. In fact, the majority of the said cytokines are found neither in the supernatant nor in the exudate. They thus remained trapped in thePRF fibrin matrix, even after serum exudation, which necessarily implies an intimate incorporation of these molecules in the fibrin polymer molecular architecture.

IGF-I is no exception. However, IGF-I is principally a circulating molecule: In the first stages of centrifugation, this cytokine is initially concentrated on the upper part of the tube, thereby explaining the high concentrations measured on that spot. On the other hand, IGF-I resulting from the platelet degranulation will certainly undergo the same metrical incorporation process as TGFb-1 and PDGF-BB. Note that the IGF-I rates resulting from cPRP technologies are necessarily low, because published quantifications are performed solely on the cPRP platelet concentrate, but, the greater part of the circulating IGF-I is in the PPP (supernatant) which is conventionally discarded during the initial cPRP production steps.

Discussion

PRF has the characteristic of polymerizing naturally and slowly during centrifugation. And the thrombin concentrations acting on the collected autologous fibrinogenare almost physiologic because there is no bovine thrombin addition.

This aspect is crucial to determine the 3-dimensional organization of a fibrin network. Indeed, during gelling, the fibrin fibrillae can be assembled between the min 2 different biochemical architectures: condensed tetramolecular or bilateral junctions and connected trimolecularor equilateral junctions.3 Bilateral junction sare constituted with strong thrombin concentrations and allow the thickening of fibrin polymers; this leads to the constitution of a rigid network, not very favorable to cytokine enmeshment and cellular migration.

However, the great resistance of such a gel is complete lyappropriate to firmly seal biologic tissues: Therefore, there will be a fibrin adhesive and, by extension, a cPRP. In contrast, weak thrombin concentrations imply a very significant percentage of equilateral junctions.

These connected junctions allow the establishment of a fine and flexible fibrin network able to support cytokines enmeshment and cellular migration (Fig. 5). Moreover, this 3-dimensional organization will give great elasticity to the fibrin matrix: It is what we observein a flexible, elastic, and very strong PRF membrane.

CONCLUSION

Although PRF belongs to a new generation of platelet concentrates, it is in the first place a fibrin technology. Indeed, the biologic activity of the fibrin molecule is enough in itself to account for the significant cicatricial capacity of the PRF. And the slow polymerization modeconfers to the PRF membrane a particularly favorable physiologic architecture to support the healing process.

However, it is now necessary to look further into platelet and inflammatory features of this biomaterial.Only a perfect understanding of its components and their significance will enable us to comprehend the clinical results obtained and subsequently extend the fieldsof therapeutic application of this protocol.

REFERENCES

1. Matras H. Die Wirkungen vershiedener fibrinpraparate aufhontinuitat-strennungen der rattenhaut. Osterr Z Stomatol 1970;67:338-59.
2. Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant?Transfusion 1990;30:741-7.
3. Mosesson MW, Siebenlist KR, Meh DA. The structure and biologicalfeatures of fibrinogen and fibrin. Ann NYAcad Sci 2001;936:11-30.
4. Clark RA. Fibrin and wound healing. Ann N Y Acad Sci 2001;936:355-67.
5. Collen A, Koolwijk P, Kroon M, van Hinsbergh VW. Influence offibrin structure on the formation and maintenance of capillaryliketubules by human microvascular endothelial cells. Angiogenesis1998;2:153-65.
6. van Hinsbergh VW, Collen A, Koolwijk P. Role of fibrin matrixin angiogenesis. Ann N Y Acad Sci 2001;936:426-37.
7. Vinazzer H. Fibrin sealing: physiologic and biochemical background.Facial Plast Surg 1985;2:291-5.
8. Whitman DH, Berry RL, Green DM. Platelet gel: an autologousalternative to fibrin glue with applications inoral and maxillofacialsurgery. J Oral Maxillofac Surg 1997;55:1294-9.
9. Saltz R, Sierra D, Feldman D, Saltz MB, Dimick A, VasconezLO. Experimental and clinical applications of fibrin glue. PlastReconstr Surg 1991;88:1005-15, discussion 1016-7.
10. Hotz G. Alveolar ridge augmentation with hydroxylapatite usingfibrin sealant for fixation. Part I: An experimental study. Int JOral Maxillofac Surg 1991;20:204-7.
11. Hotz G. Alveolar ridge augmentation with hydroxylapatite usingfibrin sealant for fixation. Part II: Clinical application. Int J OralMaxillofac Surg 1991;20:208-13.
12. Bonucci E, Marini E, Valdinucci F, Fortunato G. Osteogenic responseto hydroxyapatite-fibrin implants in maxillofacial bonedefects. Eur J Oral Sci 1997;105:557-61.
13. Matras H. Fibrin sealant in maxillofacial surgery. Developmentand indications. A review of the past 12 years. Facial PlastSurg 1985;2:297-313.
14. Matras H. Fibrin seal: the state of the art. J Oral Maxillofac Surg1985;43:605-11.
15. Soffer E, Ouhayoun JP, Anagnostou F. Fibrin sealants and plateletpreparations in bone and periodontal healing. Oral Surg OralMed Oral Pathol Oral Radiol Endod 2003;95:521-8.
16. Tayapongsak P, O'Brien DA, Monteiro CB, Arceo-Diaz LY.Autologous fibrin adhesive in mandibular reconstruction withparticulate cancellous bone and marrow. J Oral Maxillofac Surg1994;52:161-5, discussion 166.
17. Giannobile WV. Periodontal tissue engineering by growth factors.Bone 1996;19(1 Suppl):23S-37S.
18. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, StraussJE, Georgeff KR. Platelet-rich plasma: Growth factor enhancementfor bone grafts. Oral Surg Oral Med Oral Pathol OralRadiol Endod 1998;85:638-46.
19. Dugrillon A, Eichler H, Kern S, Kluter H. Autologous concentratedplatelet-rich plasma (cPRP) for local application in boneregeneration. Int J Oral Maxillofac Surg 2002;31:615-9.
20. Anitua E. The use of plasma-rich growth factors (PRGF) in oralsurgery. Pract Proced Aesthet Dent 2001;13:487-93.
21. Weibrich G, Kleis WK, Hafner G, Hitzler WE, Wagner W. Comparison of platelet, leukocyte, and growth factor levels inpoint-of-care platelet-enriched plasma, prepared using a modifiedCurasan kit, with preparations received from a local bloodbank. Clin Oral Implants Res 2003;14:357-62.
22. Arpornmaeklong P, Kochel M, Depprich R, Kubler NR, WurzlerKK. Influence of platelet-rich plasma (PRP) on osteogenic differentiationof rat bone marrow stromal cells. An in vitro study. Int JOral Maxillofac Surg 2004;33:60-70.
23. Carter CA, Jolly DG, Worden CE Sr, Hendren DG, Kane CJ.Platelet-rich plasma gel promotes differentiation and regenerationduring equine wound healing. Exp Mol Pathol 2003;74:244-55.
24. Yamada Y, Ueda M, Hibi H, Nagasaka T. Translational researchfor injectable tissue-engineered bone regeneration using mesenchymalstem cells and platelet-rich plasma: from basic researchto clinical case study. Cell Transplant 2004;13:343-55.
25. Yamada Y, Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T.Autogenous injectable bone for regeneration with mesenchymalstem cells and platelet-rich plasma: tissue-engineered boneregeneration. Tissue Eng, 2004. 10(5-6): p. 955-64.
26. Sonnleitner D, Huemer P, Sullivan DY. A simplified techniquefor producing platelet-rich plasma and platelet concentrate forintraoral bone grafting techniques: a technical note. Int J OralMaxillofac Implants 2000;15:87982.