Autologous platelet-rich plasma for topical application as regenerative therapy in dogs

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Abstract

This protocol describes a step-by-step procedure for the production of autologous platelet-rich plasma for topical application in dogs.

This protocol was used in the following publication:

Tambella AM, Attili AR, Dini F, Palumbo Piccionello A, Vullo C, Serri E, Scrollavezza P, Dupré G. Autologous platelet gel to treat chronic decubital ulcers: a randomized, blind controlled clinical trial in dogs. *Veterinary Surgery*, 43(6), 2014: 726-733. (ISSN: 0161-3499) (DOI: 10.1111/j.1532-950X.2014.12148.x).

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Protocol

Background

Step 1.

The platelet-rich plasma (PRP), or the platelet gel (PG, the coagulated form of PRP), is a hemo-component for topical use that could has an autologous or allogeneic origin. It is obtained from the aggregation of a platelet concentrate mixed with calcium and with biological (thrombin) or pharmacological aggregating factors.[1] The topical use of PRP promotes the healing process in both soft tissues[2,3] and orthopaedic conditions.[4,5] A variety of protocols and activating agents have been proposed in recent years. All the following substances are to be considered activating agents: the bovine thrombin, the agonist peptide of the thrombin receptor, the gelling agent ITA (NATREX Technologies, Inc., Greenville, NC), the batroxobin (clotting enzyme isolated from the venom of the snake *Bothrops atrox,* belonging to the Viperidae family), ascorbic acid, pulse electric field and autologous thrombin.[6-13] Over the last few years, this type of therapy has been significantly expanding in veterinary medicine.[3,4]

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Autologous whole blood collection

Step 2.

Collect autologous whole blood (50 ml) from the jugular vein into a 60mL syringe.

Add acid citrate dextrose solution (ACD-A) at a ratio of 1:9 achieving anticoagulation. ACD-A solution contains sodium citrate bihydrate 22.0 g/L, citric acid monohydrate 8.0 g/L, glucose monohydrate 24.5 g/L in sterile water for injection.

Collect additional 10 mL whole blood in two sodium citrate tubes (3.8%), to extract thrombin.

Complete blood count

Step 3.

Use a small aliquot of whole blood for complete blood cell count.

First centrifugation

Step 4.

For density separation of blood components, transfer the 50 mL specimen to a Falcon tube and spin at 180 units of gravitational force (x g) for 20 min.

First separation of blood components

Step 5.

Separate plasma and buffy coat layer and transfer in a Falcon tube under aseptic conditions in a laminar flow cabinet.

Second centrifugation

Step 6.

Spin the plasma and the buffy coat again at 650 x g for 15 min to stratify platelet concentrates (PCs, platelet pellet) in the bottom layer, and platelet poor plasma (PPP) in the supernatant layer.

Second separation of blood components

Step 7.

Discard part of the PPP, leaving in the tube 14mL volume.

Re-suspension of the solution

Step 8.

Resuspend the platelet pellet in the PPP and transfer the PRP solution into glass Petri capsule dishes. Choose number and size of the petri capsule dishes to be used based on the type of lesion and morphology of the point of application.

PRP cell count

Step 9.

Perform cellular count from PRP automatically.

Compare the mean platelet concentration in the PRP and in the whole blood.

Autologous thrombin preparation

Step 10.

Spin the whole blood collected in 5 mL sodium citrate tubes at 650 x g for 10 minutes.

Mix the plasma fraction with 10% calcium gluconate (446 mEq/L of calcium), at a ratio of 5:1, and incubate at 37°C for 30 min, in an air-jacketed CO_2 incubator.

Squash the clot obtained and collect the final supernatant, the thrombin-rich solution.

PRP activation

Step 11.

Activate the PRP by mixing in sterile glass Petri dishes the PRP, the thrombin-rich solution and the calcium gluconate (volumetric ratio 8:1:0.5) gently rotating the dish. The generation of the ready to use PRP in gel form (PG, platelet gel) should be obtained at room temperature within 5-10 minutes.

Recommendations for laboratory conditions during the production phases

Step 12.

Perform these laboratory procedures under aseptic conditions in a laminar flow cabinet following Good Laboratory Practice.

Sterility assay of the PRP product

Step 13.

Evaluate aerobic, anaerobic and fungal contaminations by bacteriological and mycological exams of the PRP product.

Topical application of the PRP

Step 14.

Separate the PG from the Petri dish using sterile tissue forceps and periosteal elevators.

Apply the PG in the target site immediately, covering the site of injury as much as possible.

REFERENCES

Step 15.

1. Decreto Ministero della Salute 3 marzo 2005.Caratteristiche e modalità per la donazione del sangue e di emocomponenti. *Gazzetta Ufficiale* n. 85 del 13 aprile 2005.
2. Carter MJ, Fylling CP, Parnell LKS. Use of Platelet Rich Plasma Gel on Wound Healing: A Systematic Review and Meta-Analysis. *Eplasty*. 2011;11:382-410.
3. Tambella AM, Attili AR, Dini F, et al. Autologous Platelet Gel to Treat Chronic Decubital Ulcers: A Randomized, Blind Controlled Clinical Trial in Dogs. *Vet Surg*. 2014;43(6):726-733.
4. Fahie MA, Ortolano GA, Guercio V, et al. A randomized controlled trial of the efficacy of autologous platelet therapy for the treatment of osteoarthritis in dogs. *J Am Vet Med Assoc*. 2013;243(9):1291-1297.
5. Gianakos A, Zambrana L, Savage-Elliott I, Lane JM, Kennedy JG. Platelet-Rich Plasma in the Animal Long-Bone Model: An Analysis of Basic Science Evidence. *Orthopedics*. 2015;38(12):e1079-e1090.
6. Landesberg R, Roy M, Glickmann RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. *J Oral Maxillofac Surg*. 2000;58(3):297-300.
7. Carter CA, Jolly DG, Worden CESr, Hendren DG, Kane CJ. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. *Exp Mol Pathol*. 2003;74(3):244-255.
8. Crovetti G, Martinelli G, Issi M, et al. Platelet gel for healing cutaneous chronic wounds. *Transfus Apher Sci*. 2004;30(2):145-151.
9. Martineau I, Lacoste E, Gagnon G. Effects of calcium and thrombin on growth factor release from platelet concentrates: Kinetics and regulation of endothelial cell proliferation. *Biomaterials*. 2004;25(18):4489-4502.
10. Leitner GC, Gruber R, Neumüller J, et al. Platelet content and growth factor release in platelet-rich plasma: a comparison of four different systems. *Vox Sang*. 2006;91(2):135-139.
11. Rughetti A, Gallo R, Caloprisco G, et al. Platelet gel: assays of three growth factors. *Blood Transfus*. 2006;4:92-101.
12. Semple E, Speck ER, Aslam R, Kim M, Kumar V, Semple JW. Evaluation of platelet gel characteristics using thrombin produced by the thrombin processing device: a comparative study. *J Oral Maxillofac Surg*. 2008;66(4):632-638.
13. Frelinger AL3rd, Torres AS, Caiafa A, et al. Platelet-rich plasma stimulated by pulse electric fields: platelect activation, procoagulant markers, growth factor release and cell proliferation. *Platelets*. 2016;27(2):128-135.