Injectable autologous platelet-rich plasma for regenerative medicine in donkeys

Adolfo Maria TAMBELLA,Evelina SERRI,Fulvio LAUS,Anna Rita ATTILI

Abstract

A step-by-step procedure for autologous platelet-rich plasma production was developed for topical percutaneous injection in donkeys.

This protocol was used in the following publication:

Faillace V, Tambella AM, Fratini M, Paggi E, Dini F, Laus F. Use of autologous platelet-rich plasma for a delayed consolidation of a tibial fracture in a young donkey. *The Journal of Veterinary Medical Science*, 79(3), 2017: 618-622. (ISSN: 0916-7250) (DOI: 10.1292/jvms.16-0400)

https://doi.org/10.1292/jvms.16-0400

https://www.jstage.jst.go.jp/article/jvms/79/3/79_16-0400/_pdf/-char/en

Citation: Adolfo Maria TAMBELLA,Evelina SERRI,Fulvio LAUS,Anna Rita ATTILI Injectable autologous platelet-rich plasma for regenerative medicine in donkeys. **protocols.io**

dx.doi.org/10.17504/protocols.io.ncbdasn

Published: 19 Feb 2018

Protocol

Background

Step 1.

The use of non-transfusional hemocomponents for tissue healing has gained increasing popularity for the treatment of musculoskeletal lesions in human and veterinary medicine [1]. Several non-transfusional hemocomponents are available for intralesional injection, including platelet-rich plasma (PRP), plasma rich in growth factors, platelet rich fibrin, platelet lysate, autologous conditioned serum, autologous blood preparations and autologous protein concentrate [2-4]. PRP is a good adjunctive therapy for the treatment of orthopedic and soft tissue conditions [5-11]. Non-unions, bone defects, tendinosis and cartilage defects are among musculoskeletal conditions lacking effective treatment modalities, and regenerative medicine may play an important role. Platelet rich plasma contains a variety of growth factors released from platelets, which increase vascular growth and have mitogenic effects on mesenchymal stem cells [12-16]. Clinical research on donkeys needs to be in continual development, since donkeys have different reactions in many conditions when compared to horses [17]. To our knowledge, PRP production and application is not commonly performed in donkeys, despite the high therapeutic potential of PRP application in this species.

This protocol was used in the following publication:

Faillace V, Tambella AM, Fratini M, Paggi E, Dini F, Laus F. Use of autologous platelet-rich plasma for a

delayed consolidation of a tibial fracture in a young donkey. *The Journal of Veterinary Medical Science*, 79(3), 2017: 618-622. (ISSN: 0916-7250) (DOI: 10.1292/jvms.16-0400)

https://doi.org/10.1292/jvms.16-0400

https://www.jstage.jst.go.jp/article/jvms/79/3/79_16-0400/_pdf/-char/en

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.

Additionally, collect 10.5 ml whole blood in 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 350 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 900 x g for 15 min to separate the platelet pellet, in the bottom layer, from the platelet poor plasma (PPP) in the supernatant layer.

Second separation of blood components

Step 7.

Discard part of the PPP, leaving in the tube 10mL volume.

Re-suspension of the solution

Step 8.

Resuspend the platelet pellet in the PPP to obtain 10 ml of PRP.

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.

To obtain the thrombin, mix the autologous plasma fraction and 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 the PRP and the thrombin-rich solution (volumetric ratio 8:1) in a Falcon tube and gently rotate the tube.

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.

Inject the PRP percutaneously in the target site, after application of routine aseptic skin preparation procedure. When necessary, the use of a guidance technique (e.g. diagnostic imaging) is recommended to accurately reach the appropriate injection site or the site of injury.

REFERENCES

Step 15.

1. Brossi, P. M., Moreira, J. J., Machado, T. S. L. and Baccarin, R. Y. A. 2015. Platelet-rich plasma in orthopaedic therapy: a comparative systematic review of clinical and experimental data in equine and human musculoskeletal lesions. *BMC Vet. Res.* 11: 98.
2. Chevalier, X. 2010. Intraarticular treatments for osteoarthritis: new perspectives. *Curr.Drug Targets* 11: 546–560.

3. Dohan Ehrenfest, D. M., Rasmusson, L. and Albrektsson, T. 2009. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol.* 27: 158–167.
4. Ziltener, J. L., Allet, L., Sclison, P. and Grosclaude, M. 2012. How effective are injections of platelet-rich plasma (PRP) for the treatment of sports injuries: a critical review of the literature. *J. Sports Medic. Doping Studie* S2: 003.
5. Boswell, S. G., Cole, B. J., Sundman, E. A., Karas, V. and Fortier, L. A. 2012. Platelet-rich plasma: a milieu of bioactive factors. *Arthroscopy* 28: 429–439.
6. Burnouf, T., Goubran, H. A., Chen, T. M., Ou, K. L., El-Ekiaby, M. and Radosevic, M. 2013. Blood-derived biomaterials and platelet growth factors in regenerative medicine. *Blood Rev.* 27: 77–89.
7. Chevalier, X. 2010. Intraarticular treatments for osteoarthritis: new perspectives. *Curr. Drug Targets* 11: 546–560.
8. Lee, K. S., Wilson, J. J., Rabago, D. P., Baer, G. S., Jacobson, J. A. and Borrero, C. G. 2011. Musculoskeletal applications of platelet-rich plasma: fad or future? *AJR Am. J. Roentgenol.* 196: 628–636.
9. Pichereau, F., Décory, M. and Cuevas, R. G. 2014. Autologous platelet concentrate as a treatment for horses with refractory fetlock osteoarthritis. *J. Equine Vet. Sci.* 34: 489–493.
10. Roukis, T. S., Zgonis, T. and Tiernan, B. 2006. Autologous platelet-rich plasma for wound and osseous healing: a review of the literature and commercially available products. *Adv. Ther.* 23: 218–237.
11. Tambella, A. M., Attili, A. R., Dini, F., Palumbo Piccionello, A., Vullo, C., Serri, E., Scrollavezza, P. and Dupré, G. 2014. Autologous platelet gel to treat chronic decubital ulcers: a randomized, blind controlled clinical trial in dogs. *Vet. Surg.* 43: 726–733.
12. Bielecki, T., Gazdzik, T. S. and Szczepanski, T. 2008. Benefit of percutaneous injection of autologous platelet-leukocyte-rich gel in patients with delayed union and nonunion. *Eur. Surg. Res.* 40: 289–296.
13. Dallari, D., Fini, M., Stagni, C., Torricelli, P., Nicoli Aldini, N., Giavaresi, G., Cenni, E., Baldini, N., Cenacchi, A., Bassi, A., Giardino, R., Fornasari, P. M. and Giunti, A. 2006. In vivo study on the healing of bone defects treated with bone marrow stromal cells, platelet-rich plasma, and freeze-dried bone allografts, alone and in combination. *J. Orthop. Res.* 24: 877–888.
14. Kawasumi, M., Kitoh, H., Siwicka, K. A. and Ishiguro, N. 2008. The effect of the platelet concentration in platelet-rich plasma gel on the regeneration of bone. *J. Bone Joint Surg. Br.* 90: 966–972.
15. Malhotra, R., Kumar, V., Garg, B., Singh, R., Jain, V., Coshic, P. and Chatterjee, K. 2015. Role of autologous platelet-rich plasma in treatment of long-bone nonunions: a prospective study. *Musculoskelet. Surg.* 99: 243–248.
16. Simman, R., Hoffmann, A., Bohinc, R. J., Peterson, W. C. and Russ, A. J. 2008. Role of platelet rich plasma in acceleration of bone fracture healing. *Ann. Plast. Surg*. 61: 337–344.
17. Laus, F., Spaterna, A., Faillace, V., Veronesi, F., Ravagnan, S., Beribé, F., Cerquetella, M., Meligrana, M. and Tesei, B. 2015. Clinical investigation on Theileria equi and Babesia caballi infections in Italian donkeys. *BMC Vet. Res.* 11: 100.