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Platelet-Rich Plasma Enhances the Cellular Function of Equine Bone Marrow-Derived Mesenchymal Stem Cells

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Abstract

Rationale: Equine bone marrow-derived mesenchymal stem cells (eBMMSCs) and platelet-rich plasma (PRP) are cell-based therapies being used clinically to repair damaged tissue of horses. Numerous reports including data from our laboratory show that there are variations in the biological properties of MSCs and platelet – rich plasma, which can impact their biological functions. A single study describes the use of the eBMMSCs and PRP in tendon healing, with minimal success. The exact mechanism of action using the combination of the two therapies is unknown. This study was performed to evaluate and understand the effects of PRP, if any on the cellular performance of eBMMSCs in culture.

Objective: To assess the effects of PRP in vitro on the rate of proliferation, expression of protein markers and osteogenic and chondrogenic differentiation of the primary cultures of eBMMSCs.

Methods and Results: A commercially available stall side, portable kit was used to isolate PRP. To investigate the effect of PRP on eBMMSCs, the rate of proliferation of eBMMSCs was measured using the MTS assay, and subsequently the viability and the stemness of eBMMSCs was assessed using fluorescent staining and the expression of CD90. Finally, the osteogenic and chondrogenic differentiation of eBMMSCs was assessed by lineage-specific staining and the expressions of lineage – specific mRNAs. All assays were performed at a concentration of 50 million platelets/mL. Significant increase in proliferation and the differentiation profiles were observed in presence of PRP. Most importantly, the stem cell characteristics of inferior eBMMSCs showed a marked improvement in presence of PRP.

Conclusions: The addition of PRP improves the in vitro function of eBMMSCs by enhancing the proliferation and osteo- and chondro- genesis. The presence of an optimal dose of platelets may enhance the in vivo performance of eBMMSCs and may be indicated when using autologous eBMMSCs therapy in the clinic.

Keywords: Equine; Mesenchymal cells; Platelet rich plasma; Platelets; Osteogenesis; Chondrogenesis

Introduction

The field of regenerative medicine is becoming an exciting area in both human and veterinary medicine that may greatly impact our ability to treat patients. Two main tools extensively being investigated are platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) and how they may aid in the treatment of orthopedic diseases. The PRP is plasma with a greater concentration of platelets than that found in whole blood, and rationale for its use arises from the release of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta (TGFβ), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and insulin-like growth factors 1 and 2 (IGF), and other cytokines, including interleukin 8. These molecules are released from the alpha granules when platelets rupture or lyse [1-5]. This process is also referred to as activation of PRP and it is these growth factors and cytokines that locally induce endogenous bone and soft tissue regeneration. Platelet-rich plasma also offers a significant advantage over other potential cell-based therapies in that it is autologous (i.e. the donor and the patient are the same), and hence, avoiding graft vs. host complications. Additionally, when PRP is activated it forms a platelet gel which can serve as an autologous scaffold which can serve as a delivery vehicle for stem cells or any other drug. This can have significant effect on some complex clinical cases for instance, wound healing. Hence, PRP is an attractive cell-based modality that can be beneficial in the treatment of various equine injuries or diseases.

The idea of providing a means for tissue to regenerate instead of repair with fibrous scar tissue is the basis for the use of MSC therapy. Therefore, the rational of combining MSCs and growth factors from PRP to synergistically enhance the regenerative processes of both therapies is reasonable. Synergistic effects of PRP and MSCs in cell-based therapies have been documented both in vitro and in vivo in human and rat models. In vitro investigations have confirmed that PRP enhances proliferation of a variety of human and rodent cell types including MSCs [6]. In vitro studies suggest that the combination of PRP and MSCs shows promise by the ability of PRP to induce the proliferation and differentiation of undifferentiated human MSCs [6-11]. Specifically, when human MSCs are grown in the presence of PRP in vitro, PRP not only enhances the MSC proliferation but also causes an increase in their osteogenic and chondrogenic differentiations [10,12].Although these studies generally yield positive results some negative outcomes in animal studies have also been reported. For example, Ogino reported that PRP enhanced the proliferation of rat osteogenic; Chondrogenesis

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bone marrow cells but suppressed their differentiation [13]. Martinello et al. reported no synergistic or additive effect of PRP and peripheral blood derived MSCs on experimentally injured deep digital flexor tendons of sheep [10].

To the best of our knowledge, there is a single study in horses where this combination therapy has been used in vivo. Torricelli et al., showed that a combination of PRP and bone marrow derived mononuclear cells resulted in marked improvement in horses with tendon damage and returned 85% of those treated with this therapy to competition. Two horses that did not return to competition had relatively lower levels of growth factor and platelet counts, suggesting, that the inefficiency of PRP may be attributed to the biological status of the animals themselves rather than to an ineffective therapy [14]. Besides, musculoskeletal defects, PRP have also been used in the treatment of equine acute and chronic full thickness cutaneous wounds, again with mixed and controversial results [15-17].

Similar to as described above for PRP, in vitro and in vivo variations in the proliferation and differentiation of MSCs alone have also been demonstrated in mice, dog, human and equine donors [18-21]. These variations can be due to the source of MSCs, age-dependent or independent donor-to-donor variation, and are further complicated when cells are expanded by passaging in culture. We recently observed significant variation in the biological properties of equine bone marrow-derived MSCs (eBMSCs) among age and gender matched horses [19]. Using molecular and cellular assays laid out by the International Society for Cellular Therapy [22], we reported that the eBM MSC population of cells generated from a 12 year old mare exhibited a higher rate of proliferation, enhanced ability for cell passaging, and relatively more robust patterns of in vitro differentiation. Comparatively, eBM SCs from 2 other age and gender – matched donors demonstrated a lower rate of proliferation and lack of osteogenic and chondrogenic differentiation [19]. The clinical significance of these findings is unknown but suggests that they could potentially affect the treatment outcome. Thus, it is important to note that not all MSCs and PRP are equivalent and the quality of these must be documented and compared to improve the treatment outcome.

Due to the demand for autologous MSC therapy, we wanted to investigate ways of enhancing the performance of the eBMSCs specifically on those cells that demonstrate poor proliferation and differentiation, as previously reported by our group. In view of the published literature, we hypothesized that the response of eBMSCS would differ when cultured with an optimal concentration of PRP. This response will be assessed in the rate of proliferation and osteo- and chondrogenic differentiation. Our main objective was to evaluate the effects of PRP if any, on the cellular function of eBMSCs. Therefore, in this current study we exploited the properties of PRP to enhance the proliferation and differentiation of eBMSCs. We performed this in focus of both the clinical and basic research in MSC therapies. To maintain consistency in the PRP preparations, for PRP isolation we selected the ProTec system from PulseVet (PulseVet, Alpharetta, GA) based on cost, portability, and ease of use. We evaluated the platelet concentration, white blood cell concentration, pH, and the effect of PRP on eBMSCS cultured from five age and gender-matched horses. We selected equine donors from the group used previously [19]. Donor 1 is the donor from which the MSCs demonstrated superior in vitro properties whereas, eBM MSCs generated from donor 5 demonstrated relatively a lower rate of proliferation and reduced osteogenic and chondrogenic potentials.
platelets.

All experiments were carried out in triplicate in 96-well plates with 3 × 10^4 cells seeded in 200 µL as the seeding volume. For the assay, 40 µL of the MTS reagent was added to each well and incubated for 3 h at 37°C/5% CO2. The optical density of the complex was measured on a microplate fluorescence reader at 490 nm. Each medium combination without cells was used as the corresponding blank. Each sample absorbance was corrected by the reference blank reading. Graph of absorbance at 490 nm versus days of proliferation was generated, and data were analyzed.

Following the results of the dose response experiment the proliferation assay was repeated with the optimal deduced platelet concentrations for eBMMSCs cultured from the 3 remaining donors (donors 2-4) as described above.

**Cell adhesion, viability, and immunofluorescence**

Equine bone marrow-derived MSCs were seeded with 50 million platelets/mL (5 fold above baseline), that was deduced as the optimal concentration in PRP. Cell adhesion and viability was assessed at day 6 using calcein-AM (Invitrogen, Eugene, OR) and propidium iodide (PI) (Invitrogen, Carlsbad, CA). Cells were stained according to the manufacturer's protocols and subsequently visualized using an electronic camera (Nikon DS-Fi2, Japan) connected to a Zeiss microscope and evaluated with the NIS Elements imaging software (Nikon).

The expression of CD90, a reliable protein marker for MSCs [26,27] was assessed using immunofluorescence. Equine bone marrow-derived MSCs were seeded in growth medium containing 50 million platelets/mL, and were incubated at 37°C and 5% CO2 for 6 days. At day 6, MSCs were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 (Sigma®) for 10 min, at room temperature, and blocked with 5% normal serum for 30 min, at room temperature. Cells were washed and incubated overnight with 5 µg/sample of primary antibody against FITC-conjugated anti-rat CD90 (BD Pharmingen®) at 4°C. After washing with HBSS buffer, cells were mounted with Slowfade® Gold antifade with DAPI reagent (Molecular Probes®) and images were obtained using an electronic camera (Nikon DS-Fi2, Japan) connected to a Zeiss microscope and evaluated with the NIS Elements imaging software (Nikon).

**Activation of PRP and bi-lineage differentiation**

For all differentiation assays, tissue culture dishes were coated with a uniform layer of activated PRP. To form a PRP gel, an appropriate concentration in PRP was used to coat each well of a 24 well plate using a micropipette. Plates were incubated at 37°C for 15 min and 1.0 × 10^7 eBMMSCs were seeded in each well for differentiation. Using PRP-coated tissue culture dishes eliminated the need of large volumes of PRP at each media change and ensured that the eBMMSCs were exposed to PRP throughout their growth and differentiation phase.

Osteogenesis and chondrogenesis were performed using standard procedures [19] with P3 eBMMSCs in presence and absence of PRP. Equine BM MSCs were seeded in presence of the complete growth medium. Seventy to eighty percent confluent cells were induced to differentiate into each of the 2 lineages. Equal numbers of cells without any differentiation media were used as controls.

Osteogenic differentiation was induced in complete growth medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 0.25 mM ascorbic acid. The medium was changed every 3 days, and differentiation was monitored using alizarin red staining after 21 days.

Chondrogenic differentiation was induced in complete growth medium supplemented with 100 nM dexamethasone, 0.25 mM ascorbic acid, and 5 ng/mL TGFβ1. The medium was changed every 3 days, and differentiation was monitored using alizarin blue staining after 14 days.

All differentiation profiles were assessed via cell-specific staining and by assessing the expression of lineage-specific mRNAs. Images were acquired with an electronic camera (Nikon DS-FI2, Japan) connected to a Zeiss microscope and evaluated with the NIS Elements imaging software (Nikon).

**RNA isolation and real time polymerase chain reaction (qPCR)**

RNA isolation and qPCR from P3 eBMMSCs in presence of PRP were performed. The total RNAs from undifferentiated cells in presence of PRP (control) and differentiated cells in presence of PRP were isolated. For RNA isolation, cells were harvested using 5 mL of Accutase (Cell detachment solution in PBS) and incubated at 37°C/5% CO2 for 30 min. Total RNA was extracted using an RNasey Mini RNA kit according to the manufacturer's instructions. One microgram of total RNA was reversing transcribed in a 10 µL reaction volume for cDNA synthesis (Biorad, Hercules, CA).

Alkaline phosphatase (ALP) was used as an osteogenic marker and, cartilage oligometric matrix protein (COMP) was used as markers for chondrogenic differentiation. Messenger RNA levels for all the markers were quantified using mRNA expression normalized with that of equine GAPDH mRNA as the internal control. Real time PCR was carried out using the absolute SYBR Green ROX quantitative PCR mix on the Mx3005P and data were analyzed using MxPro software (Agilent Technologies, Santa Clara, CA). Cq values representing the level of each mRNA expression were calculated. Using a cDNA blank, samples with an undetectable gene expression level (i.e., a Ct value of ≥ 30) were given an arbitrary expression level of zero. The relative expression of each target gene was calculated as ACt, Delta Cq values for each mRNA under a specific condition for each donor were compared. The expressions were compared between undifferentiated and differentiated samples in presence of PRP.

**Statistical Analysis**

All data are expressed as the mean ± standard deviation. All analyses were done using a combination of STATA 12 and SPSS 22. Normality of the data was first confirmed using the Shapiro-Wilke test and then analyses were performed using the paired, Student t-test. P<0.05 was considered statistically significant for all analyses.

**Results**

**Characterization of PRP**

Roughly 5 mL of PRP was obtained in one preparation from each of the five equine donors using the ProTec kit. Each horse was sampled in duplicate. The platelet counts in the whole blood ranged from 1.7 to 2.3 × 10^6/mL with a mean value of 1.9 × 10^6/mL. Comparatively, platelet
counts in PRP ranged from 2.7 to 4.3 × 10⁸/mL with a mean value of 3.5 × 10⁸/mL. Therefore, each preparation resulted in 1.5-1.9 fold enrichment in the platelets compared to the whole blood. Each PRP preparation contained negligible amount (<1%) of white blood cells. The pH of each PRP sample was 7.2-7.3. For all experiments the culture media was standardized to 10 million platelets/mL. This is referred to as the baseline group. The TGF-b1 and PDGF-BB concentrations in the PRP were 3309.63 ± 1036.27 pg/mL and 2989.23 ± 561 pg/mL, respectively.

**PRP enhances the rate of proliferation of eBMMSCs**

To determine an optimal platelet concentration a dose-response experiment was carried out to assess the effect of varying amounts of platelets on the rate of proliferation of eBMMSCs from two horses with contrasting MSC properties (donors 1 and 5 from the earlier study). Cell proliferation was evaluated in presence and absence of PRP using MTS assays after days 2 and 6 (Figures 1 and 2). Cells grown in the absence of PRP served as the control. At each time point, there was a general increase in cell proliferation with increasing concentration of platelets above the baseline. The 5-fold concentration of platelets had the largest and the most consistent significant effect in both the donors and at both time points, and hence, was chosen as the optimal dose. This observation was further confirmed in eBMMSCs isolated from the remaining three equine donors 2-4 (Figure 3). Collectively, a 3.5 to 3.7 fold increase in cell proliferation on day 2 and a 2.2-3.0 fold increase in cell proliferation on day 6 were observed. PPP treatment groups equivalent to that of PRP were assayed for each donor and data showed that the effect on cell proliferation is solely due to platelets in the PRP (data not shown).

**The cell viability and stemness is maintained in presence of PRP**

Based on proliferation experiments we cultured eBMMSCs in presence of 5 fold PRP for all further experiments. The tissue culture dishes were coated with a volume which contained 5 fold platelet dose. Cell adherence and viability was tested using calcein-am/PI live-dead

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**Figure 1**: Measurement of rate of proliferation of eBMMSCs. The cell proliferation assay was used to assess the rate of proliferation of eBMMSCs from donor 1 over a period of 6 days, as a function of platelet concentration in PRP. The rates were obtained in presence of 10 million platelets/mL (baseline), 1.5, 3, 5 and 7.5 fold above the baseline and were compared to the rates of proliferation of cells grown in the absence of PRP (control). The single dagger symbol and the asterisk demonstrate a significant value with at least p<0.005.

**Figure 2**: Measurement of rate of proliferation of eBMMSCs. The cell proliferation assay was used to assess the rate of proliferation of eBMMSCs from donor 5 over a period of 6 days, as a function of platelet concentration in PRP. The rates were obtained in presence of 10 million platelets/mL (baseline), 1.5, 3, 5 and 7.5 fold above the baseline and were compared to the rates of proliferation of cells grown in the absence of PRP (control). The dagger symbols and the asterisk demonstrate a significant value with at least p<0.005.
staining after 6 days. Positive staining of eBMMSCs with calcein-am (green) and lack of PI staining (red) of eBMMSCs demonstrate that cells adhere and are viable in presence of PRP. A representative image is shown (Figure 4). Additionally, the expression of CD90 was confirmed when the eBMMSCs were cultured in presence of 5 fold PRP (Figure 5).

PRP promotes osteo- and chondrogenesis in vitro

Since the MTS assay and calcine-am/PI staining demonstrated a positive effect of PRP on the viability and rate of proliferation of eBMMSCs from the 5 donors tested, we next investigated the effect of PRP on the osteogenic and chondrogenic potentials. In vitro differentiation patterns were evaluated in eBMMSCs in presence of 5 fold PRP. As expected greater than 90% cells were positive for CD90.

Osteogenic differentiation of eBMMSCs was investigated after PRP stimulation at day 7. Osteogenesis was assessed qualitatively by alizarin red staining (Figure 6). Alizarin red staining increased significantly in differentiated cells compared to the control (undifferentiated) group in presence of PRP, suggesting an increase in the osteogenic capacity of eBMMSCs in presence of PRP.

Chondrogenesis of eBMMSCs in presence of PRP was assessed at day 7 by alcian blue staining. Results similar to those described above for osteogenesis were seen including an increase in cell stain uptake in the differentiated cells cultured with PRP when compared to the control (undifferentiated) group (Figure 6).

Interestingly, the eBMMSCs from donors which failed to show any osteo- or chondro- differentiation in the absence of PRP (described in our earlier study by Carter-Arnold et al. [19]) demonstrated a robust stimulation at day 7. Osteogenesis was assessed qualitatively by alizarin red staining (Figure 6). Alizarin red staining increased significantly in the differentiated cells compared to the control (undifferentiated) group in presence of PRP, confirming the increased differentiation potentials of eBMMSCs in presence of PRP.

Discussion

In a previous study [19] from our laboratory, we established cultures of eBMMSCs from 6 middle-aged mares. The results of this study demonstrated that cultures of eBMMSC from these donors proliferated at different rates and were able to differentiate in varying degrees into osteocytes and chondrocytes. Two out of six eBMMSC cultures showed minimal to no osteo- and chondrogenic differentiation, and had significantly lower rates of proliferation. Since, eBMMSCs from all donors satisfied the criteria to be classified as MSCs [22,26], i.e. the cells adhered to the polystyrene surface, expressed the CD44 and CD90 proteins and were isolated and expanded using standard cell culture methods, we were confident that these are indeed MSCs with relatively inferior biological properties and hence if used in therapy, these cells may not prove to be efficient in repairing bone and cartilage tissues. The eBMMSCs from one donor, on the other hand might prove to be very useful and might result in significant improvement if they are used in a bone or cartilage injury. This same idea of intrinsic variables affecting
eBMMSCs quality and function was also hypothesized for PRP content profiles and was recently investigated by Giraldo et al. [28]. In this study the authors demonstrated that breed, age, and gender all influenced the growth factor concentrations in pure PRP and PRP gel. Hence, a detailed analysis of eBMMSCs and PRP is imperative. With this in view, in the present study, we evaluated the PRP cellular and growth factor profiles achieved by a commercial kit and determined if the addition of exogenously added growth factors in the form of PRP would have an effect on eBMMSCs in culture. We hypothesized that since PRP is an enriched reservoir of growth factors, it would rescue some of the biological properties (in vitro proliferation and differentiation) of eBMMSCs from all donors with a specific interest in those previously showing a lack of differentiation.

A stall-side, portable, single use commercial PRP kit was used to obtain consistent preparations of PRP and PPP samples from all donors. All samples demonstrated a 1.5-1.9 enrichment in platelets with <1% leukocytes, constituting this commercial kit as a leukocyte reduced source of PRP. Additionally, growth factor concentrations between all donors were within the ranges that have been previously reported with no statistical difference noted between any donors. A significant dose of 50 million platelets/mL (P<0.05) did result in increased proliferation of eBMMSCs from all donors tested (Figures 1A-C). Cell viability assays

![Figure 6: Osteogenesis and chondrogenesis differentiation assays.](image)

**Figure 6:** Osteogenesis and chondrogenesis differentiation assays. In vitro osteocyte differentiation (top panel) of eBMMSCs from donors 1–5. Images depict osteocyte differentiation in the presence of PRP. Mineralized nodules were visible as cells differentiated into osteocytes. Cells were fixed and stained red with Alizarin red on day 7 post differentiation. The lower panel depicts undifferentiated (control) cells in presence of PRP. Note the lack of mineralized nodules in the control cells. The insets shown in this figure demonstrate the differentiation in the absence of PRP. These data are adapted from the report published earlier [19]. Scale bar=100 μm. In vitro chondrocyte differentiation (bottom panel) of eBMMSCs from donors 1–5. Images depict chondrocyte differentiation in the presence of PRP. Cell aggregates were visible as cells differentiated into chondrocytes. Cells were fixed and stained red with Alcian blue on day 7 post differentiation. The lower panel depicts undifferentiated (control) cells in presence of PRP. Note the lack of cell aggregates in the control cells. The insets shown in this figure demonstrate the differentiation in the absence of PRP. These data are adapted from the report published earlier [19]. Scale bar=100 μm. Note the significant improvement in the osteocyte and chondrocyte differentiation patterns in presence of PRP.

![Figure 7: mRNA expression assays.](image)

**Figure 7:** mRNA expression assays. Quantitative PCR was performed to compare the expression of ALP and COMP in undifferentiated (control) and differentiated eBMMSCs from donors 1–5 in presence of PRP. Relative expression of ALP and COMP mRNAs is shown. The Ct values for each target is normalized with GAPDH mRNA. Fold change in differentiated cells was calculated in comparison to the undifferentiated cells for each target gene. Asterisk demonstrates significant data with p<0.05. Each qPCR was repeated with at least two independent biological samples with n=3-6 for each RNA sample/target mRNA. Note the significant increase in the expression of each of these targets after differentiation.
also confirm that the presence of platelets in the culture medium is not deleterious to cell proliferation. Most importantly this data shows that there is a marked enhancement of proliferation of eBMMSCs which could not grow in the absence of PRP, suggesting that the platelets can improve the proliferation ability of eBMMSCs. Additionally, the positive expression of a reliable MSC marker protein, CD90 in all eBMMSCs generated in this study confirmed that eBMMSCs retained their stemness in presence of PRP.

In this study equine PRP exerted a positive osteogenic and chondrogenic stimulatory effect on eBM MSCs in vitro in all donors based on gene expression and cell specific staining (Figures 6 and 7). This was strongly supported by lineage-specific staining in addition to the changes in osteocyte and chondrocyte specific mRNA expression patterns. Significant increases (P<0.05) in ALP, Sox9 and COMP mRNAs in the presence of PRP confirm that eBM MSCs from all donors have the potential to differentiate, some, however due to variations in their biological properties, might require a cell-based factor i.e. platelets to help them undergo differentiation. Noteworthy, is the observation that eBM MSCs from donor 5 did not undergo an efficient osteo- and chondro-genesis in the absence of PRP, whereas they demonstrated marked differentiation-specific staining and increase in lineage-specific differentiation patterns in presence of PRP.

Similar to other published studies we also observe that this effect on cell proliferation and differentiation is dependent on the platelet concentration. Lennon and group directly related proliferation and differentiation of rat MSCs to platelet concentration and it began at a 4-5 fold increase above baseline [29]. Choi et al. showed that the proliferation of alveolar bone cells was suppressed at high and were stimulated at low PRP concentrations [30]. As a result of our dose response curve in all our experiments a 50 million platelet/mL concentration was used. Our dose was selected based on the MTS day 6 data indicating 75 million/mL concentration to have a decrease in cell proliferation when compared to the 50 million/mL concentration. Whether this is the optimal dose in vivo needs to be established.

The effect of PRP on the eBM MSCs in this study may be attributed to the cytokines and growth factors of platelets, however, it is still unknown whether the stimulatory effects of PRP is related to the growth factors or to other microparticles from the cytoplasm or cell membrane that are released from activated platelets [31]. Platelets release several growth factors such as TGF beta 1 and PDGF type BB, which can exert anti-inflammatory, anabolic, and angiogenic effects, and future experiments are required to assess the exact mechanism and identify the protein targets involved in this process.

The results of the current study confirm that PRP has a stimulatory effect on eBM MSCs proliferation and differentiation in vitro in horses. The presence of PRP does not affect the viability and the expression of cell surface marker proteins in eBM MSCs. It is most important that we were able to restore the function of eBM MSCs previously classified as poor quality demonstrated by improved osteo and chondrogenesis. Utilizing PRP with eBM MSCs that demonstrate poor proliferation and differentiation may be indicated for both in vitro culturing conditions and at local injection. This positive effect will hopefully improve the in vivo function of eBM MSCs and can be used to enhance the application of PRP and MSC – based therapies.

Conclusions

In conclusion, the in vitro results of this study demonstrate that the proliferation of equine BM MSCs can be improved by the presence of PRP, which can subsequently enhance their potential bone and cartilage formation abilities. The in vivo application of these data however, needs to be confirmed. These findings are important for further studies towards technological advances in equine regenerative medicine.

Acknowledgements

This work was supported by grants from the University of Tennessee (UT) Center of Excellence in Livestock Diseases & Human Health (MD). The authors also acknowledge the generous donation of Adrian Lock, Pulsevet, ProTec for providing us with the PRP kits.

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