

In vitro study: ESWT treatment of platelets causes increased release of growth factors.

Introduction: Platelet-rich plasma (PRP) and extracorporeal shockwave therapy (ESWT) are both used for the treatment of soft tissue injuries in horses. Clinically, the question has been raised whether these two therapies could be used in combination. The hypothesis of this study was the application of ESWT to PRP would increase the release of platelet derived growth factors (PDGF) and transforming growth factors beta 1 (TGF β) from platelets.

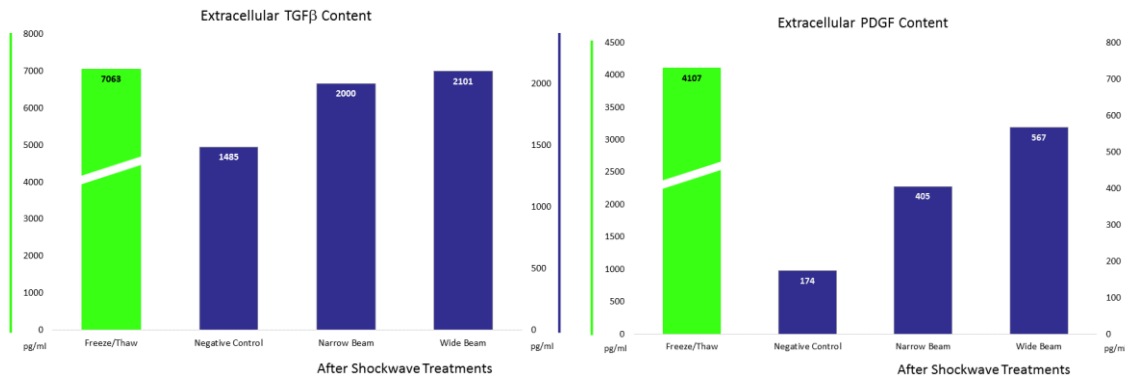
A pilot study was performed which evaluated an array of settings on the shockwave device, including frequency settings (Hz or pulses per minute), energy settings, probe type and number of pulses. This pilot study revealed to us that a lower frequency setting as well as higher energy had greatest benefit. The data pertaining to the number of shocks suggested that more shocks yielded a greater biologic response. The pilot data was used to determine the settings used in the study protocol.

Methods: The horses used to produce PRP from were all Quarter Horse mares aged 11-15 years. Aliquots of PRP were injected into a gel pad designed to mimic the acoustic properties of soft tissue. The gel pads were submerged into a water bath for coupling purposes and exposed to ESWT simulating a treatment depth of 25mm. Treatments consisted of 300 pulses at 23kV and 2Hz with two probes (i) a 20mm wide beam and (ii) a 10mm narrow beam (Figure 1).

Focus Length	Width	Energy Density	Total Energy
Wide Beam	20 mm	0.12 mJ/mm ²	8.5 mJ/pulse
Narrow Beam	10 mm	0.23 mJ/mm ²	4.4 mJ/pulse

Figure 1

The supernatant from the PRP sample was then collected. Growth factor concentrations were quantified using ELISA kits for platelet-derived growth factor, isoform BB (PDGF-BB) and transforming growth factor β , isoform 1 (TGF β). The samples treated with ESWT were compared to both a positive control (freeze/thaw cycle) and standard negative control. The freeze/thaw cycle was chosen as the positive control because it is known to provide the best PRP activation.



Results: The TGF-β concentrations from the freeze/thaw cycle (positive control) were significantly higher than all other treatments. Both ESWT treatment groups resulted in significantly greater increase in TGF-β1 concentration compared to the negative controls. The wide probe and the narrow probe resulted in 41.5% and 34.6% increase in growth factor concentration, respectively.

The PDGF-BB concentrations from the freeze/thaw cycle were significantly higher than all other treatments. Both ESWT treatment groups resulted in significantly greater increase in PDGF-BB concentration compared to the negative controls. The wide probe and the narrow probe resulted in 226.4% and 133.5% increase in growth factor concentration, respectively.

Conclusion: The results of this study supported our hypothesis. Release of growth factors from PRP were significantly increased following treatment with extracorporeal shockwave therapy when compared to untreated growth factor concentrations (negative control). This data supports the use of ESWT immediately following therapeutic injection of PRP into injured soft tissue structures in the horse to increase the concentrations of growth factors released from the platelets.

The data favors the use of a wide beam shockwave source with medium energy density (mJ/mm^2) with a high dose (mJ/pulse).