Mechanisms of Action of Pulsed Electromagnetic Field Therapy on a Rat Model of Rotator Cuff Injury and Repair

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Introduction

Rotator cuff tears affect millions of individuals each year, often requiring surgical intervention. Although advancements in surgical and rehabilitation protocols have improved clinical results, rotator cuff repair failure is common.[1] To improve surgical outcomes, non-invasive therapies have been utilized post-operatively.[2] We have previously shown that pulsed electromagnetic field (PEMF) therapy improved tendon-to-bone mechanical properties in a rat model of rotator cuff injury and repair,[3,4] consistent with increased type I collagen and fibronectin protein expression and increased collagen alignment,[4] potentially providing an explanation for the improved mechanical properties. However, these alterations in composition and tissue structure could be downstream of specific physiological responses to PEMF treatment, including changes in inflammation, cell signaling, cell metabolism, increased production of matrix components, and/or changes in matrix degradation and remodeling.[5] Therefore, the objective of this study was to determine the influence of PEMF treatment on tendon gene expression and cell composition during early stages of healing. We hypothesized that PEMF treatment would amplify tendon-healing related signaling pathways such as TGF-β while mitigating inflammation.

Methods

106 adult male Sprague-Dawley rats (400-450g) were used (IACUC approved). Animals underwent acute supraspinatus injury and repair[3] followed by systemic exposure to Physio-Stim® PEMF (Orthofix, Inc.) for 1 hour daily. Control animals did not receive PEMF therapy (non-PEMF). Animals were euthanized at 3, 7, 14, 21, or 28 days post-op (n = 10/group/time point). From half of the animals, right supraspinatus tendons were dissected out and divided into insertion and midsubstance portions for RNA isolation, cDNA synthesis, specific target amplification, and Fluidigm qPCR for 40 target genes and 2 housekeeping genes (n = 5/group/time point). Expression was normalized to the housekeeping genes and then to non-PEMF at each time point. From the other half of the animals, right shoulders were dissected and processed for histological analysis. H&E stained sections were semi-quantitatively graded for cell density (cellularity) and cell shape[3,4] (n = 5/group/time point), and CD68 and CD163 immunohistochemical staining was performed for M1 and M2 macrophages, respectively (n = 4/group at 14 and 28 days). Statistical comparisons were made between PEMF and non-PEMF groups over time and at each time point, using two-way ANOVAs with Bonferroni post-hoc tests. Immunohistochemical staining was qualitatively assessed in a blinded manner.

Results

Gene expression

Expression of the BMP2 signaling molecule was increased with PEMF treatment in the tendon insertion across time (Fig 1A); downstream targets collagen type 1a (Fig 1B), alkaline phosphatase (Fig 1C), and osteocalcin (Fig 1D) were also upregulated with PEMF. Although transforming growth factor (TGF) β1 and 2 were unchanged (data not shown), expression of TGFβ3 was downregulated with PEMF treatment in the tendon insertion (Fig 1E). Matrix metalloproteinase (MMP) 9 and connective tissue growth factor (CTGF) were upregulated early and downregulated late (Fig 1F,G), and MMP13 was downregulated across time (not shown). Fibronectin expression increased in PEMF treated tendons (Fig 1H). Similar expression patterns of TGFβ3, MMP9, and fibronectin were seen in the tendon midsubstance (not shown). Expression of inflammatory markers was also altered with PEMF, including increased interleukin-10 and tachykinin (Fig 2A,B), and decreased interleukin-1β and tumor necrosis factor α (Fig 2C,D).

Immunohistochemistry

At 14 days, CD68+ (M1) macrophages were increased in the midsubstance of non-PEMF tendons (Fig. 2E, top panel). No differences were seen at 28 days, or in the insertion (not shown). CD163+ (M2) macrophages were increased in the insertion of PEMF tendons at 14 days (Fig 2E, bottom) with no differences seen at 28 days or in the midsubstance (not shown).

Histology

There were no differences in cell density or cell shape in either the tendon insertion or

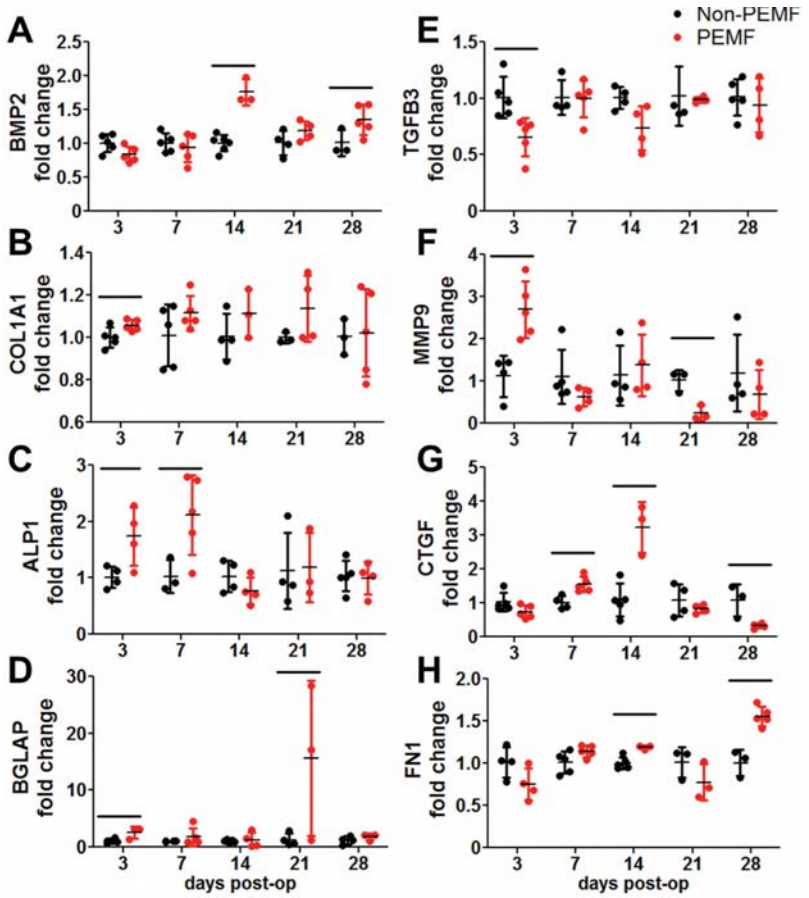

Figure 1. BMP2 & TGFβ Related Gene Expression. At the tendon insertion, PEMF treatment **(A)** increased *Bmp2*, **(B)** increased *Col1a1*, **(C)** increased *Alp1*, **(D)** increased *Bglap*, **(E)** decreased TGFβ3, **(F)** altered *Mmp9*, (G) increased *Ctgf*, and increased (F)*Fn1*. Data shown as mean±SD, normalized to housekeeping and then normalized to non-PEMF at each time point (n = 5/group/time point).

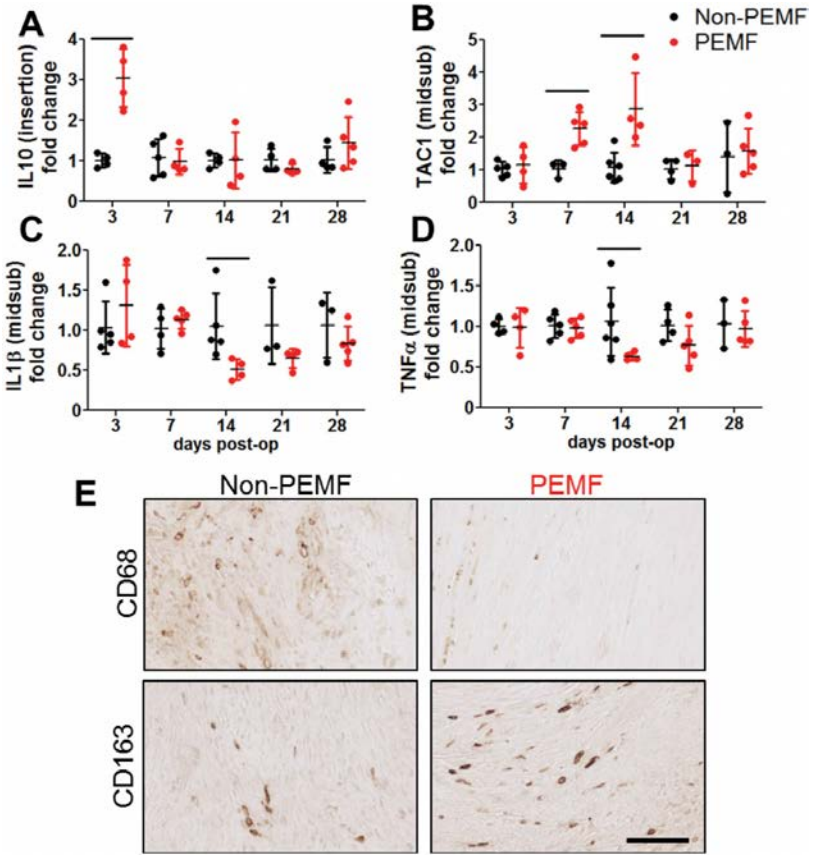

Figure 2. Inflammation. Gene expression of inflammatory markers were altered with PEMF treatment, including **(A)** increased *Il10*, **(B)** increased *Tac1*, **(C)** decreased *Il1*β, and **(D)** decreased *Tnf*α. Representative 200x images show **(E, top)** decreased CD68+ staining at the midsubstance and **(E, bottom)** increased CD163+ staining at the insertion at 14 days post-op in PEMF tendons. Scale bar: 100μm.

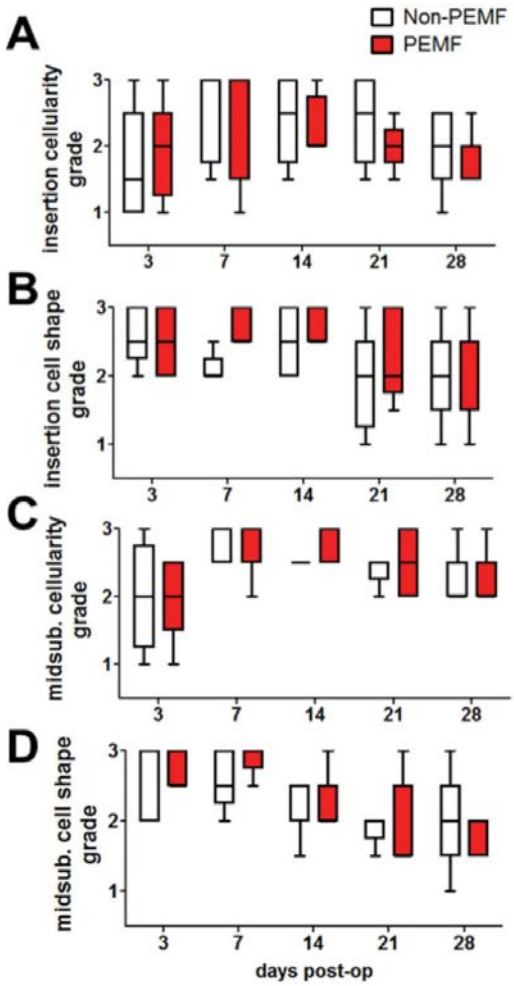

Figure 3. H&E Histological Properties. No differences were found between groups for **(A)** insertion cellularity, **(B)** midsubstance cellularity, **(C)** insertion cell shape, or (D) midsubstance cell shape. Midsubstance properties were altered over time. Data shown as median+IQR.

midsubstance with PEMF treatment compared to non-PEMF controls (Fig 3). Cell shape and cellularity varied over time in the midsubstance for both groups but did not significantly change in the insertion.

Discussion

This study demonstrated molecular and cellular changes within supraspinatus tendons after injury with PEMF treatment. Gene expression data suggests an upregulation in the BMP2 signaling pathway, including increased collagen production during early healing. Increases in pro-osteogenic genes at the insertion could support important processes to re-establish the tendon-bone interface. Future work will assess kinetic bone properties in the greater tuberosity. Decreased TGFβ3 and changes in MMP expression support a downregulation in the fibrotic response with PEMF, which coincides with a decreased tendon cross-sectional area at 4 weeks seen in previous studies.[4] Interestingly, PEMF had a consistent anti-inflammatory effect, upregulating *Il10* and *Tac1*, and downregulating IL1β and TNFα, as well as a decrease in CD68+ macrophages and increase in CD163+ macrophages at 14 days in PEMF- treated tendons. Similar mechanisms have been shown in intervertebral disc cells after PEMF treatment *in vitro* [5] and *in vivo*.[6] Although statistical comparisons were not made, regional differences in gene expression and cell morphology support the need to assess tendon responses regionally.

Significance

Previous work showing improved rotator cuff healing with PEMF supported the initiation of a PEMF clinical trial for this condition. This study provides important mechanistic insight into how PEMF affects cellular and molecular processes in the supraspinatus tendon after injury.

Acknowledgements

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