# Role of Pulsed Electromagnetic Fields (PEMF) on Tenocytes and Myoblasts—Potential Application for Treating Rotator Cuff Tears

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**ABSTRACT:** The post-surgery integrity of the tendons and muscle quality are the two major factors in success of rotator cuff (RC) repair. Though surgical techniques for rotator cuff repair have significantly improved in the past two decades, there are no effective treatments to improve tendon-to-bone healing and muscle quality after repair at this point in time. Pulsed electromagnetic fields (PEMF) have previously been used for promoting fracture healing. Previous studies have shown that PEMF has a positive role in promoting osteoblast precursors proliferation and differentiation. However, PEMFs effect on tenocytes and muscle cells has not been determined fully yet. The purpose of this study is to define the role of a commercially available PEMF on tenocytes and myoblasts growth and differentiation in vitro. Human rotator cuff tenocytes and C2C12 murine myoblasts were cultured and treated with PEMF for 2 weeks under regular and inflammatory conditions. Our results showed that 2 weeks treatment of PEMF enhanced gene expressions of growth factors in human rotator cuff tenocytes under inflammatory conditions. PEMF significantly enhanced C2C12 myotube formation under normal and inflammatory conditions. Results from this study suggest that PEMF has a positive role in promoting tenocyte gene expression and myoblast differentiation. Therefore, PEMF may potentially serve as a non-operative treatment to improve clinical incomes rotator cuff tendon repairs. Results © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 35:956–964, 2017.

**Keywords:** rotator cuff tear; tenocyte myoblast; PEMF

Rotator cuff tears are extremely common shoulder injuries. The prevalence of full-thickness rotator cuff (RC) tears is reported as 21% in the general population and increases considerably with aging.[1] Surgical repair for torn tendons remains the most definitive treatment option for symptomatic RC tears when conservative management fails. In US alone, 75,000 patients undergo rotator cuff surgery annually.[2] Small and medium sized tears are amenable to surgical repair, while massive tears often cause significant impairment of shoulder function, and have worse surgical incomes.[3] Outcomes following surgical repair are predicated on both tendon healing and improved muscle function. Clinical studies have demonstrated that the integrity of the tendons and muscle quality following repair are the two major factors in success of rotator cuff repair.[1,4]

Secondary, pathological changes of rotator cuff muscles following RC tears include muscle atrophy[5–7] and fatty infiltration,[8–11] which result in decreased joint function even after successful tendon repairs. The presence of these changes in RC muscles have been correlated with higher rates of re-tear following intervention. More importantly, the development of muscle atrophy and degeneration after RC tears is often thought to be clinically irreversible.[5,12–16] Though surgical techniques for RC repair have significantly improved in the past two decades, there are still no effective treatments to improve muscle quality after RC tears surgical repair. Further, despite improved biomechanical constructs, there are few solutions for biologically improving tendon to bone healing for RC repairs.

Pulsed electromagnetic field (PEMF) therapy, a safe biophysical adjunct treatment, has been demonstrated to have beneficial therapeutic effects on a variety of bone related disorders. The FDA has approved several PEMF devices, which provide a complementary solution to spinal fusions[17,18] in addition to improving healing of non-unions and delayed unions.[19] Though the detailed mechanism is not fully understood, previous studies have shown that PEMF promotes osteoblast precursor proliferation and differentiation,[20–22] as well as stem cell osteogenesis.[23,24] The success of PEMF in promoting bone healing has boosted the enthusiasm of expanding its use for treating other tissue injuries. Recent studies have shown positive effect of PEMF in promoting wound healing and cartilage repair.[25,26] Recently, Osti et al. reported a randomized controlled trial with patients undergoing rotator cuff repair which showed that the application of pulsed electromagnetic fields after rotator cuff repair is safe and reduces postoperative pain, analgesic use, and stiffness in the short term.[27] However, the underline mechanism of the effect of various PEMF on rotator cuff tendon and muscle remains unknown. In this study, we examined the effect of Physio-Stim®, an FDA-approved PEMF, on primary human rotator cuff tenocytes, and a myoblast cell line—C2C12 cells. We hypothesized that PEMF would be safe technique to enhance tenocyte and myoblast differentiation, which

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may suggest a role for PEMF in improving outcomes after RC repair.

## MATERIALS AND METHODS

### Human Rotator Cuff Tenocyte Harvest

Rotator cuff tissue was harvested from one healthy 19-year-old male donor obtained from Allosource Inc. (Centennial, CO). The donor's supraspinatus tendon was dissected into 5 mm × 5 mm pieces. Explants of tissue were then plated in sterile Falcon™ tissue culture dishes (Fisher Scientific Inc., Pittsburgh, PA) and cultured in standard cell culture media (DMEM with 10% fetal bovine serum and 1% antibiotic-antimycotic) at 37°C in a humidified atmosphere containing 5% $CO_2$. Tenocytes were allowed to expand from the explants and were collected when reaching 80% confluence. Tenocytes were then passaged for three to five passages before being plated into 6-well Falcon™ tissue culture plates with a density of 320,000 cells/well.

### Myoblast Culture

C2C12 murine myoblasts (American Type Culture Collection, Rockville, MD) were seeded at a density of 160,000 cells/well in 6-well Falcon™ tissue culture plates. The C2C12 cells were cultured with standard cell culture media (DMEM with 10% fetal bovine serum and 1% antibiotic-antimycotic) at 37°C in a humidified atmosphere containing 5% $CO_2$.

### Simulating Inflammatory Condition With IL-1

To simulate the inflammatory conditions, the cells were cultured with standard medium supplemented with 10 ng/ml interleukin(IL)-1α (R&D system, Minneapolis, MN) as described previously.[28] The medium was changed every other day with freshly made IL-1α containing medium. For all experiments, cells were cultured for 1 and 2 weeks without passaging.

### PEMF Treatment

The 6-well Falcon™ tissue culture plates containing tenocytes or myoblasts were placed in the center of $CO_2$ incubator equipped with a Physio-Stim® PEMF system (Orthofix Inc., Lewisville, TX). Cells were exposed to PEMF for 3 h a day throughout the experiment. Cells in the control group were placed in the same incubator with the PEMF system turned off.

### Cell Viability

Cell viability was analyzed using the LIVE/DEAD Viability/Cytotoxicity kit (Life Technologies Inc. Carlsbad, CA). Live/dead assay was performed at 1 and 2 weeks after PEMF treatment. Live cells were stained with a 50 μM calcein AM solution, while dead cells were stained with 2 mM ethidium homodimer solution according to the manufacture's instruction. Cells were then sorted via a Fluorescence Activated Cell Sorting (FACS) AriaII system (B.D. Biosciences Inc., Franklin Lakes, NJ).

### Myoblast Histology and Fusion Index

C2C12 cells were fixed by 4% paraformaldehyde (PFA) for 15 min at room temperature. Cells were then blocked with 2% bovine serum albumin in 1xPBS for 30 min and incubated with anti-myosin monoclonal antibody (Sigma–Aldrich, St. Louis, MO) at 1:1,000 dilution for 2 h. After washing, cells were then incubated with anti-mouse IgG 650 secondary antibody (Li-Cor Inc., Lincoln, NE) at 1:2,000 dilution for 1 h. The nuclei were counterstained with 1:10,000 DAPI (Thermo Fisher Scientific Corp., Waltham, MA) for 5 min. The cells were then were imaging with Carl Zeiss AG microscope (Carl Zeis Inc. Oberkochen, Germany). C2C12 myogenesis was determined by fusion index, which was calculated by dividing the total number of nuclei within multinucleated myotubes by the total number of nuclei as described previously.[29] Eight replicates per condition were counted. Images were reviewed and fusion index was the average of calculation made by two independent reviewers who were blinded to the experiment.

### Reverse Transcriptase–Polymerase Chain Reaction (RT–PCR)

Total RNA for both tenocytes and myobytes was isolated using Trizol reagent (Life Technology, Inc.) according to manufacturer's instructions. cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Applied Bioscience, Indianapolis, IN). Real-time PCR was performed to quantify the expression of interested genes using SYBR Green Detection and an Applied Biosystems Prism 7900HT detection system (Applied Biosystems, Inc., Foster City, CA). Genes and sequences of the primers used for myoblasts and tenocytes were summarized in Tables 1 and 2, respectively. Gene expression level was normalized to the internal control of S26 for myoblasts and GAPDH for tenocytes. Fold changes relative to non-PEMF controls were calculated using $\Delta\Delta C_T$.

### Statistical Analysis

All results were presented as mean ± standard deviation. *T*-test was used for comparison between PEMF treated and no PEMF treated control groups in each condition at each time point. Significant difference was considered when $p < 0.05$.

**Table 1.** Sequence of Murine Genes Primers for C2C12 PCR Experiment

| Genes | Forward Primer Sequence | Reverse Primer Sequence |
|---|---|---|
| Myomaker | CAG TGA GCA TCG CTA CCA AGA G | GAA TGT CAC GGC GCA TGA AGC A |
| MyoD | ATC CGC TAC ATC GAA GGT CT | CGC TGT AAT CCA TCA TGC CA |
| MHC1 | GAG ATT TCT CCA ACC CAG | TCT GAC TTT CGG AGG TAC T |
| MHC2a | ATG AGC TCC GAC GCC GAG | TCT GTT AGC ATG AAC TGG TAG GCG |
| MHC2b | GTG ATT TCT CCT GTC ACC TCT C | GGA GGA CCG CAA GAA CGT GCT GA |
| MHC2x | AAG GAG CAG GAC ACC AGC GCC CA | ATC TCT TTG GTC ACT TTC CTG CT |
| Myostatin | CAG TGA ATG CAA CTC CCA CA | CGA GCA AAT GAT CTC CTG GG |
| Myogenin | TTA CGT CCA TCG TGG ACA GC | TGG GCT GGG TGT TAG CCT TA |
| RPS26 | CGG AAC ATT GTA GAA GCC GCT G | CCT TGC TAT GGA TGG CAC AGC T |

**Table 2.** Sequence of Human Genes Primers For Rotator Cuff Tenocytes PCR Experiment

| Genes | Forward Primer Sequence | Reverse Primer Sequence |
|---|---|---|
| TGFβ-1 | GCAACAATTCCTGGCGATACC | AAAGCCCTCAATTTCCCCTCC |
| TGFβ-3 | CTAAGCGGAATGAGCAGAGGATC | TCTCAACAGCCACTCACGCACA |
| bFGF | GGCTTCTTCCTGCGCATCCA | GCTCTTAGCAGACATTGGAAGA |
| IGF-1 | TGGATGCTCTTCAGTTCGTG | TGGTAGATGGGGGCTGATAC |
| PDGF-B | GAGATGCTGAGTGACCACTCGA | GTCATGTTCAGGTCCAACTCGG |
| Scleraxis A | AGAACACCCAGCCCAAACA | TCGCGGTCCTTGCTCAACTT |
| Tenomodulin | TTGAAGACCCACGAAGTAGA | ATGACATGGAGCACACTTTC |
| BMP-2 | GGGCATCCTCTCCACAAA | GTCATTCCACCCCACGTC |
| BMP-12 | GCAGCCGCTGTCTCCGCCTC | TCTGCGTCGTTAAGGCTGGACA |
| BMP-13 | TGCCAGC2TTTTTCCAGTCTT | AGGAGTGTGCGAGAGATCGT |
| BMP-14 | AACAGCAGCGTGAAGTTGGAGG | ACACGTACCTCTGCTTCCTGAC |
| MMP-2 | AGCGAGTGGATGCCGCCTTTAA | CATTCCAGGCATCTGCGATGAG |
| MMP-3 | ATTCCATGGAGCCAGGCTTTC | CATTTGGGTCAAACTCCAACTGTG |
| MMP-13 | TCCCAGGAATTGGTGATAAAGTAGA | CTGGCATGACGCGAACAATA |
| MMP-14 | CCCAACATCTGTGACGGGAACT | GAGCAGCATCAATCTTGTCGGTAG |
| TIMP-4 | GAGAGTGTCTGCGGATACTTC | GCAGGTAGTGATGTGCAAGAGTC |
| COL1A | CAA TGC TGC CCT TTC TGC TCC TTT | CAC TTG GGT GTT TGA GCA TTG CCT |
| COL3A | TAT CGA ACA CGC AAG GCT GTG AGA | GGC CAA CGT CCA CAC CAA ATT CTT |
| GAPDH | CGA CAG TCA GCC GCA TCT TCT TT | ACC AAA TCC GTT GAC TCC GAC CTT |

## RESULTS

### PEMF Did Not Affect Tenocyte and Myoblast Cell Viability

FACS-based live/dead assay demonstrated that tenocytes viability was $99.9 \pm 0.2\%$ and $99.8 \pm 0.3\%$ in PEMF-treated and control groups after 1 week and $99.9 \pm 0.1\%$ and $99.7 \pm 0.1\%$ in PEMF-treated and control groups after 2 weeks of treatment (Fig. 1). No significant difference was found between the treated and control groups at 1 week time point ($p = 0.55$) and 2 week time point ($p = 0.37$). Similar results were found in myoblast viability assays. After 1 week of PEMF treatment, $97.9 \pm 1.3\%$ of myoblast remained alive. There was no significant difference when compared to that in the control group, in which $95.3 \pm 1.7\%$ cells remained alive ($p = 0.11$) (Fig. 2). After 2 weeks PEMF treatment, $87 \pm 4.5\%$ of myoblasts remained alive, with no significant difference compared to that in the non-treated control group ($88.5 \pm 6.3\%$) ($p = 0.76$).

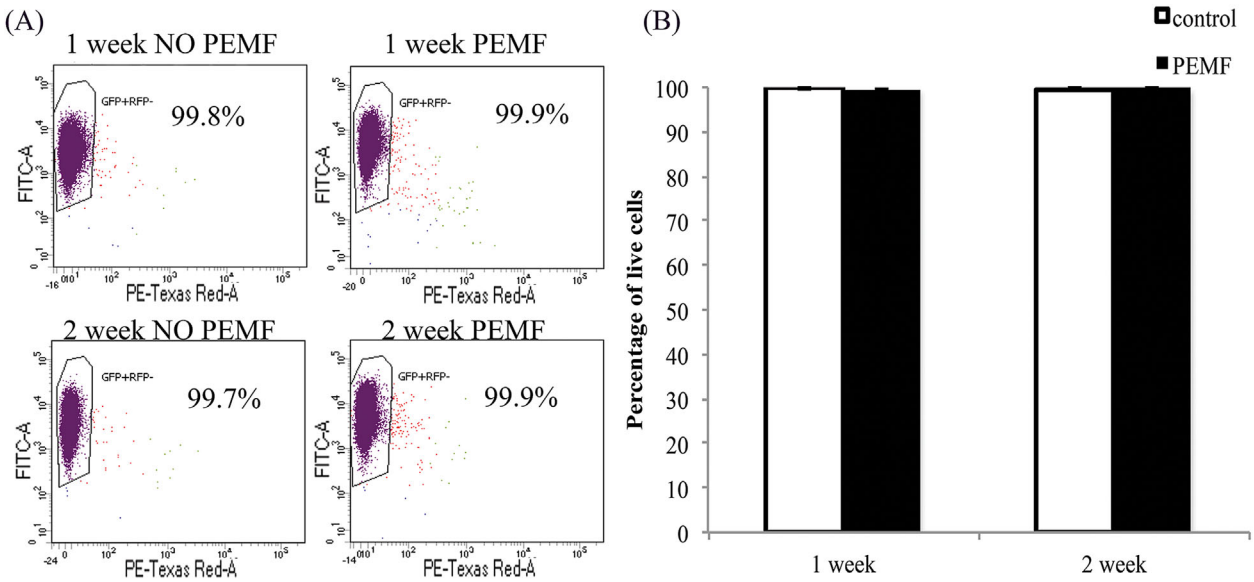


**Figure 1.** The results of live and dead assay for tenocytes with/without PEMF treatment. (A) Representative FACS result of tenocyte live/dead assay. Purple dots represent live cells (GFP+ RFP−), green dots represent the dead cells and the red dots represent uncertain cells (blue dots represents cell debris). Areas of live cells are highlighted in each figure. There is no significant difference between PEMF-treated and control groups at either time point ($N = 3$).

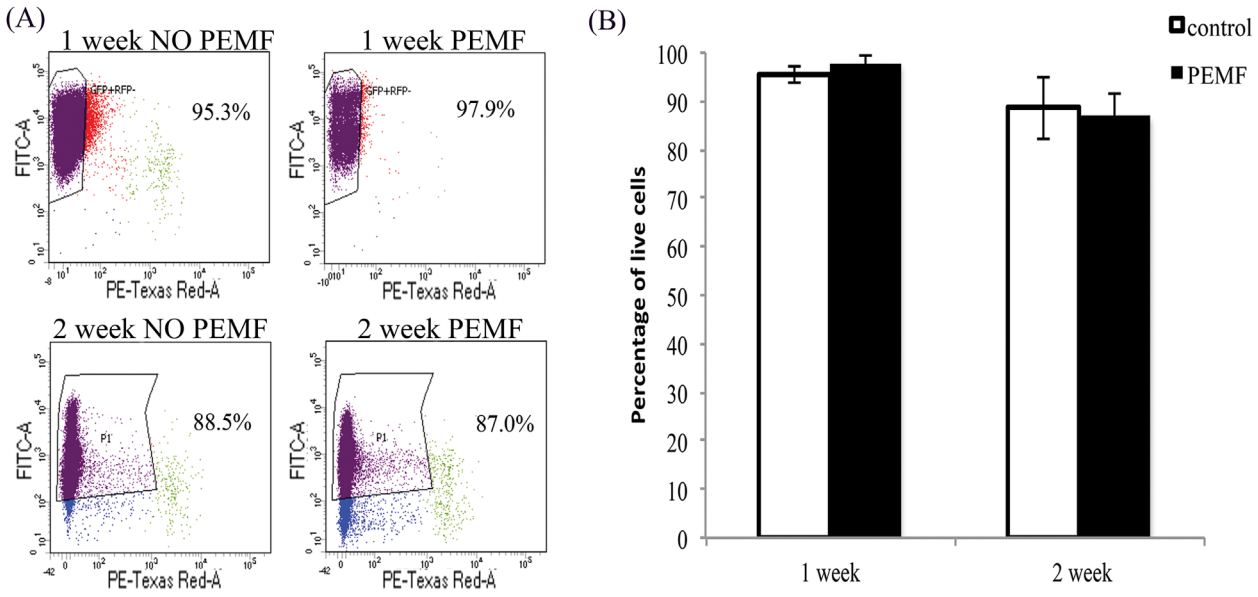


**Figure 2.** The results of live and dead assay for C2C12 myoblast with/without PEMF treatment. (A) Representative FACS result of tenocyte live/dead assay. Purple dots represent live cells (GFP+ RFP−), green dots represent the dead cells and the red dots represent uncertain cells (blue dots represents cell debris). Areas of live cells are highlighted in each figure. (B) Quantification of percentage of live cells/total cells in each group at 1 and 2 weeks after treatment. There is no significant difference between PEMF-treated and control groups at either time point ($N = 3$).

### PEMF up-Regulated Myogenic Gene Expression

In standard medium, 1 week of PEMF treatment did not alter the expression level of myogenic genes in C2C12 cells compared to non PEMF treatment control. However, prolonged treatment of PEMF for 2 weeks significantly increased myomaker and myosin heavy chain 1 (MHC1) gene expression levels by 2.3- and 4.7-folds, respectively in C2C12 myoblasts compared to control groups ($p < 0.05$ for both genes) (Fig. 3).

Under inflammatory condition (10 ng/ml of IL-1 in standard medium), PEMF regulates C2C12 myoblast gene expression with a different pattern. Similar to that in standard medium, 1 week treatment of PEMF did not alter the expression level of key myogenesis genes in C2C12 cells. However, prolonged treatment of PEMF for 2 weeks significantly increased MyoD expression for 9.4-fold ($p < 0.05$), but did not alter the expression level of myomaker or MHC1 expression compared to the control (Fig. 4).

### PEMF Increased Myotube Formation

Histology demonstrated more myotubes formed by C2C12 myoblasts in standard medium in the PEMF-treated group compared to the control group (Fig. 5). After 1 week, the fusion index was $76.25 \pm 1.20\%$ in the PEMF-treated group and $67.75 \pm 3.32\%$ in the control group. At 2 weeks, the fusion index for PEMF group is $94.00 \pm 1.41\%$ and $82.25 \pm 2.76\%$ in the control group. At both time points, PEMF significantly

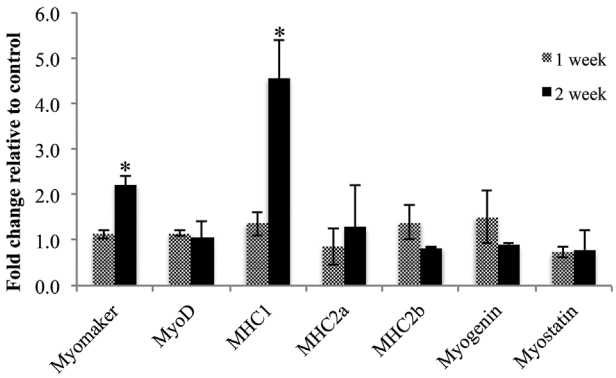


**Figure 3.** The Effect of PEMF on myoblast gene expression under standard culture condition. With 2 weeks of PEMF treatment, the myomaker and MHC1 expression increased 2.3- and 4.7-folds compared to control for C2C12 cells ($N = 6$) ($^*p < 0.05$).

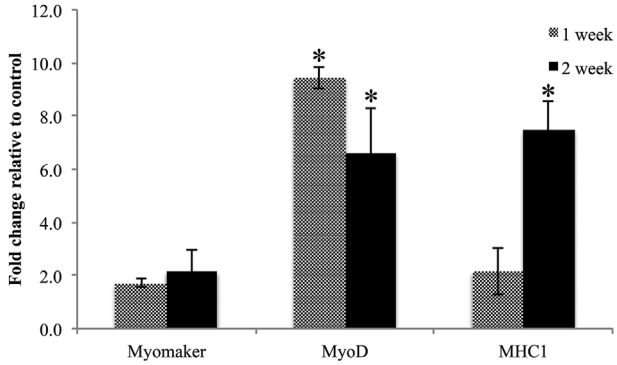


**Figure 4.** Effect of PEMF on myoblast gene expression under pro-inflammation conditions. Compared to the non-treated control, 2 weeks treatment of PEMF significantly up-regulated MyoD gene expression (9.4-fold) in C2C12 myoblasts compared to the control group ($N = 6$) ($^*p < 0.05$).

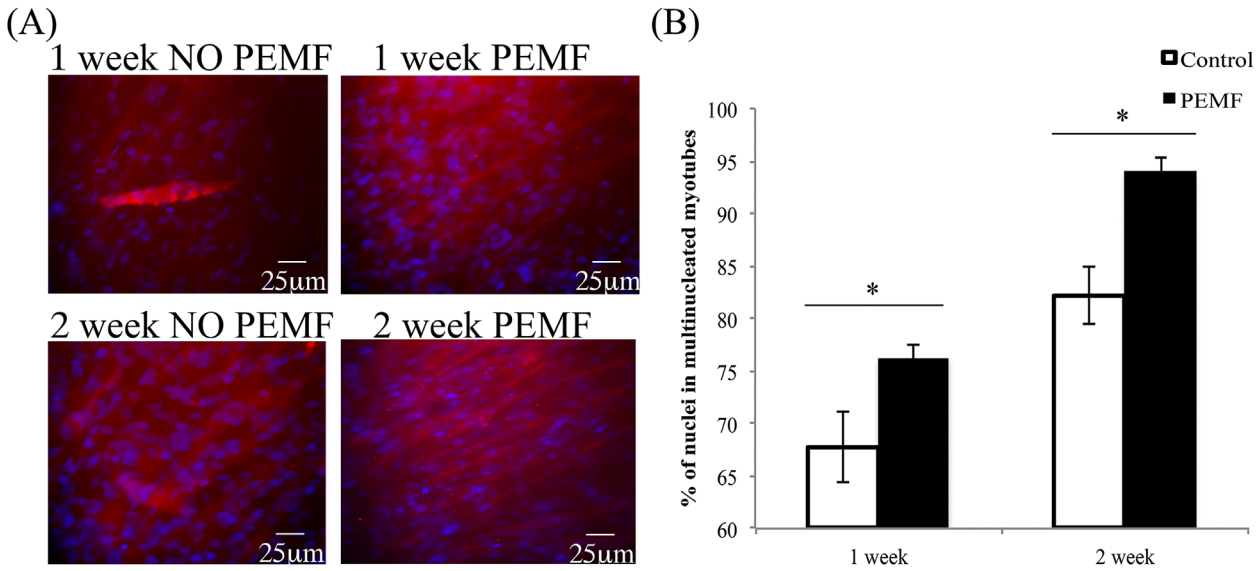


**Figure 5.** IHC of myotube formation in standard media with and without PEMF (A) Typical images of C2C12 mytube formation in PEMF-treated and control groups at 1 and 2 weeks after treatment. Myotube was stained with anti-myosin antibody (red) and nuclei were counter stained with DAPI (blue). (B) Quantification of fusion index (percentage of myotube nuclei/total nuclei) in PEMF-treated and control groups at 1 and 2 weeks under standard media. PEMF significantly increased fusion index in C2C12 cells at both time points ($N = 8$) ($^*p < 0.05$).

increased myotube fusion index in C2C12 myoblasts compared to the control group ($p < 0.05$) (Fig. 5B).

The effect of PEMF on C2C12 myotube formation was more significant when the cells were treated with IL-1. Under pro-inflammatory conditions (10 ng/ml of IL-1), the fusion index was $23.0 \pm 6.9\%$ in the PEMF-treated group and $6.7 \pm 3.9\%$ in the control group. At 2 weeks, the fusion index for PEMF group is $72.9 \pm 11.0\%$ and $37.2 \pm 10.2\%$ in the control group (Fig. 6). At both time points, PEMF significantly increased myotube fusion index in C2C12 ($p < 0.05$) (Figure 6B).

### PEMF Increased Tenocyte Gene Expressions Under Inflammatory Condition

Under standard culture condition, PEMF did not significantly change the expression of any genes tested in tenocytes at either 1 or 2 weeks after treatment (Fig. 7). However, under inflammatory conditions (10 ng/ml of IL-1), PEMF treatment significantly increased the gene expression of collagen I (2.9-fold) at 1 week and growth factors of TGFβ-1 (3.5-fold), PDGFβ (6.3-fold), and BMP12 (3.0-fold), as well as TIMP4 (5.1-fold) at 2 weeks in human rotator cuff tenocytes ($p < 0.05$) (Fig. 8).

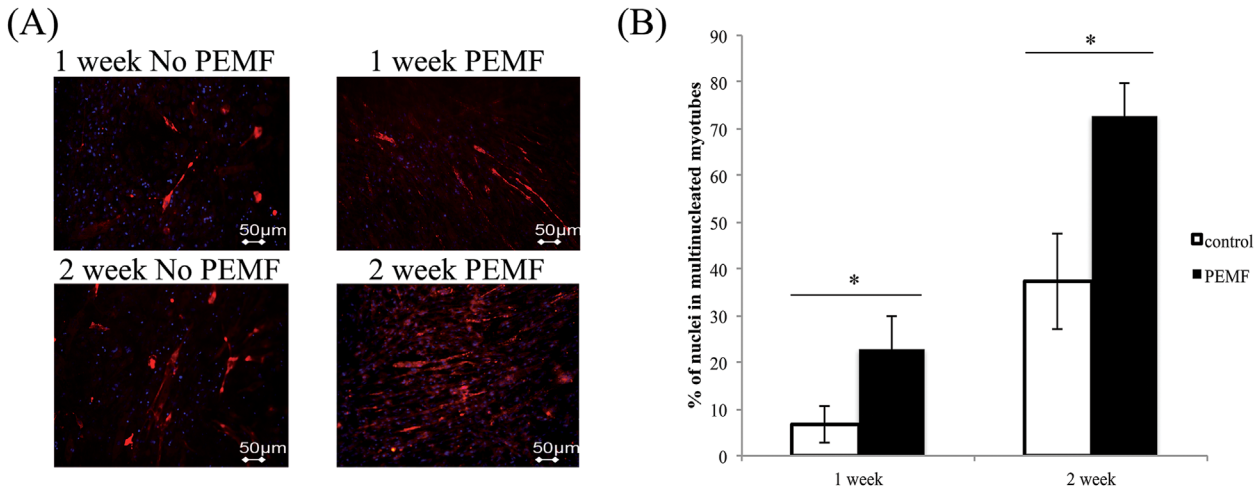


**Figure 6.** IHC of myotube formation under inflammatory conditions with and without PEMF. (A) Typical images of C2C12 mytube formation in PEMF-treated and control groups at 1 and 2 weeks after treatment. Myotube was stained with anti-myosin antibody (red) and nuclei were counter stained with DAPI (blue). (B) Quantification of fusion index (percentage of myotube nuclei/total nuclei) in PEMF-treated and control groups at 1 and 2 weeks under inflammatory conditions. PEMF significantly increased fusion index in C2C12 cells at both time points ($N = 8$) ($^*p < 0.05$).

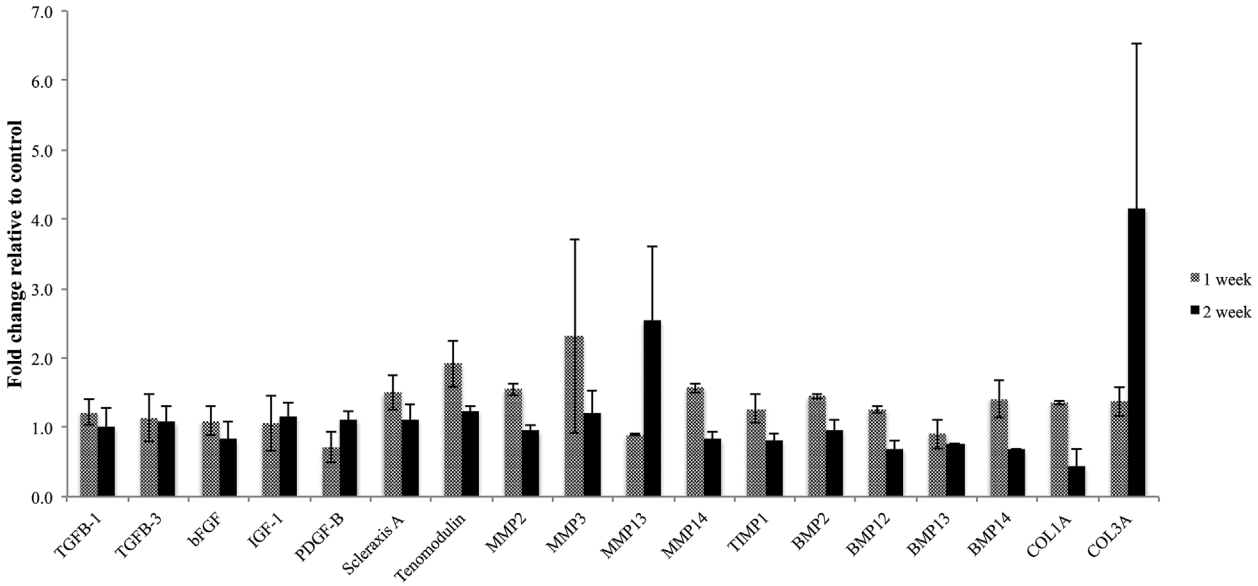


**Figure 7.** Gene expressions of human rotator cuff tenocytes cultured in standard media with or without PEMF. PEMF treatment did not alter the expression level of any gene within the panel at either 1 or 2 weeks after treatment compared to non-treated control ($N = 6$).

## DISCUSSION

The goal of this in vitro study was to examine the effect of Physio-Stim®, an FDA-approved PEMF, on the viability and differentiation of primary human rotator cuff tenocytes and a myoblast cell line. We hypothesized that PEMF would be safe technique to enhance tenocyte and myoblast viability and differentiation. We found that PEMF did not jeoparize tenocyte and myoblast cell viability at both time points examined. We found that PEMF resulted in a marked increase in myotube formation with a concomitant rise in pro-myogenic gene expression. Although PEMF did not stimulate tenocyte gene expression under normal conditions, it did increase the factors associated with tenocyte growth and differentiation in inflammatory conditions which may mimic the postoperative state.

Tendon disorders are common and are responsible for morbidity in many orthopedic patients. Tendon healing can occur intrinsically, via proliferation of epitenon, and endotenon tenocytes. Internal tenocytes contribute to the intrinsic repair process by secreting collagen fibers and stimulating tendon to bone healing.[30] The effect of PEMF on tendon repair is not well investigated, and previous reports have demonstrated

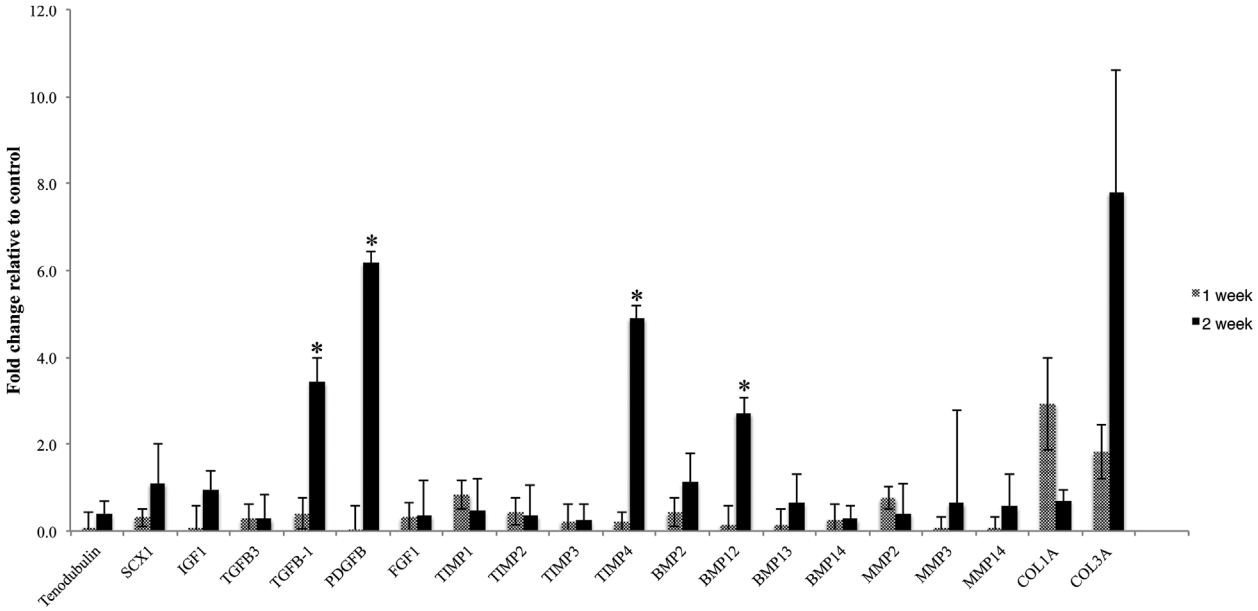


**Figure 8.** Gene expressions of human rotator cuff tenocytes cultured in pro-inflammation condition with or without PEMF. Compared to non-treated control, PEMF treatment significant up-regulated Collagen I expression at 1 week and TGFβ, PDGFβ, TIMP4, and BMP12 at 2 weeks after treatment ($N = 6$) ($^{*}p < 0.05$).

contradictory results.[31–34] These controversial findings may be due to in part to the disparate source of tenocytes. Thus, we used primary human rotator cuff tenocytes in this study.

In this in vitro study, treatment with PEMF did not significant alter gene expression of collagens, extracellular matrix components, growth factors, MMPs, and other molecules in standard culture medium. Our results are consistent with a previous study using primary cultured tenocytes from human supraspinatus and quadriceps tendons, in which PEMF was found to have no effect on tenocyte growth, cell cycle, and total collagen accumulation.[35] This suggests that our current PEMF signal may not have a substantial role in stimulating RC tenocyte gene expression under the normal culture condition.

However, when tenocytes are cultured in a pro-inflammatory environment, PEMF significantly promoted gene expression of type I collagen, certain growth factors, including TGF-β, PDGFβ, and BMP-12 (also known as Growth differentiation factor 7, GDF7) and TIMP-4, an important regulator of a group of extracellular matrix remodeling enzymes—matrix metalloproteinases (MMPs) in tenocytes. Previous studies have also showed that although PEMF had no effect on tenocyte growth and collagen accumulation, it significantly accelerated tenocyte healing after injury.[35] This interesting result suggests that tenocytes cultured under different physiological conditions react to PEMF in different way. Though PEMF has minimal effect on gene expression of tenocytes cultured in standard media, it has a beneficial role in promoting their gene expressions under an inflammatory condition. Given the pro-inflammatory post-surgical state, this provides preliminary data suggesting that PEMF may have a beneficial role in stimulating factors associated with tendon to bone healing in a post-surgical state.

Large and massive rotator cuff tears are usually accompanied with muscle atrophy and degeneration. However, there is no effective treatment for these muscle changes at this time. A myoblast is an embryonic progenitor cell that differentiates to muscle cells in a process known as myogenesis.[36] Though myoblast proliferation and fusion into myofibers usually happens during developmental stage, recent studies suggest it can still occur in the adult following injury or other more subtle stresses.[36–38] Promotion of myogenesis of myoblasts and their derivatives, including satellite cells, may therefore be a promising countermeasure for muscle atrophy and degeneration after RC tears.[39] In our study, we tested the role of PEMF on myoblast myogenesis. Our result demonstrated that PEMF increased fusion index of myoblast 2–5-folds compared to non-treated controls under both standard and inflammatory conditions. Though the underlining mechanism of PEMF function remains undiscovered, this result suggests that PEMF may serve as non-invasive treatment to promote muscle regeneration during RC repair, as well as after other muscle injuries.

Our finding that PEMF promotes C2C12 myogenesis is consistent with a previous study with a dual-mode electric and magnetic biological stimulator (EM-Stim). In this study, the authors found that combined electric and magnetic fields accelerated myogenic differentiation of myoblasts into myotubes.[40] Electric and magnetic fields are both present with PEMF. However, it remains unknown which is more important in promoting myogenesis. Future studies are needed to fully discover the mechanism of PEMF-induced myogenesis of myoblasts, and to determine other important factors such as exposure duration and field strength in optimizing these promising early results.

Inflammation has been proven to be an important factor in tendon to bone healing. However, the effect of inflammation on rotator cuff muscle degeneration after RC tears remains unclear. Previous studies have demonstrated the presence of macrophages in RC muscle after tendon tears in both human and animal studies.[41,42] Furthermore, inhibition of 5-LOX, COX-1, and COX-2 increases tendon healing and reduces muscle degeneration after RC repair in rats.[43] These results suggest that inflammation also plays an important role the development of secondary muscle pathological changes after RC tears. Interestingly, PEMF was more effective in promoting C2C12 myogenesis under inflammatory conditions compared to standard culture conditions. This data suggests that PEMF may be more suitable to treat muscle atrophy and degeneration associated with inflammation.

There are several limitations of this study. First, only one PEMF treatment strategy (daily treatment with 3 h a day) was tested in this study. This is the same strategy currently FDA approved for PEMF to treat fracture non-unions. However, the optimal treatment time and frequency of PEMF to treat non-union may be different from that to treat RC tears. Future work is needed to test different PEMF strategies on muscle and tendon cells. Secondly, the primary cultured human tenocytes used in this study were cultured for third to fifth passage before experiments. Previous study suggests that tenocytes from human Achilles tendon start to lose partial phenotype as early as the third passage,[44,45] Though possible phenotype drift of cultured rotator cuff tenocytes has not been demonstrated up to date, it is possible that the cultured rotator cuff tenocytes may possess slightly different phenotype than those in situ. Thirdly, we only evaluated the role of PEMF on gene expression of tenocytes and myoblasts. Future work should explore the cellular and molecular mechanisms by evaluating the protein expression level that govern the changes that occur due to PEMF, as this understanding will help delineate the use of PEMF clinically. Last but not least, only in vitro experiments were included in this study. Future in vivo animal studies

are clearly needed to fully evaluate the role of PEMF in treating RC tears.

In summary, our findings in this study suggest that PEMF has a significant effect on myoblasts and tenocytes, and could potentially serve as a safe and effective measure to promote tendon integrity and muscle regeneration after rotator cuff injuries.

## AUTHORS' CONTRIBUTIONS

Mengyao Liu performed analysis on samples, interpreted data, wrote manuscript. Carlin Lee and Dominique Laron helped with analyzing the samples and interpreted data. Nianli Zhang, Erik I. Waldorff, and James T. Ryaby helped to edit the manuscript. Brian Feeley and Xuhui Liu supervised development of experiments, helped in data interpretation and edit the manuscript. Xuhui Liu also helped with drafting the manuscript and acted as corresponding author. All authors have read and approved the final submitted manuscript.

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## REFERENCES

1. Yamamoto A, Takagishi K, Osawa T, et al. 2010. Prevalence and risk factors of a rotator cuff tear in the general population. J Shoulder Elbow Surg 19:116–120.
2. Vitale MA, et al. 2007. Rotator cuff repair: an analysis of utility scores and cost-effectiveness. J Shoulder Elbow Surg 16:181–187.
3. Kim JH, et al. 2014. Retear rate in the late postoperative period after arthroscopic rotator cuff repair. Am J Sports Med 42:2606–2613.
4. Vastamaki M, Lohman M, Borgmastars N. 2013. Rotator cuff integrity correlates with clinical and functional results at a minimum 16 years after open repair. Clin Orthop Relat Res 471:554–561.
5. Goutallier D, et al. 2003. Influence of cuff muscle fatty degeneration on anatomic and functional outcomes after simple suture of full-thickness tears. J Shoulder Elbow Surg 12:550–554.
6. Nakagaki K, et al. 1996. Fatty degeneration in the supraspinatus muscle after rotator cuff tear. J Shoulder Elbow Surg 5:194–200.
7. Barton ER, et al. 2005. Rat supraspinatus muscle atrophy after tendon detachment. J Orthop Res 23:259–265.
8. Zanetti M, Gerber C, Hodler J. 1998. Quantitative assessment of the muscles of the rotator cuff with magnetic resonance imaging. Invest Radiol 33:163–170.
9. Goutallier D, Postel JM, Bernageau J, et al. 1994. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. Clin Orthop Relat Res 78–83.
10. Coleman SH, et al. 2003. Chronic rotator cuff injury and repair model in sheep. J Bone Joint Surg Am 85-A: 2391–2402.
11. Safran O, et al. 2005. Changes in rotator cuff muscle volume, fat content, and passive mechanics after chronic detachment in a canine model. J Bone Joint Surg Am 87:2662–2670.
12. Gladstone JN, et al. 2007. Fatty infiltration and atrophy of the rotator cuff do not improve after rotator cuff repair and correlate with poor functional outcome. Am J Sports Med 35:719–728.
13. Gerber C, Fuchs B, Hodler J. 2000. The results of repair of massive tears of the rotator cuff. J Bone Joint Surg Am 82:505–515.
14. Gerber C, et al. 2004. Effect of tendon release and delayed repair on the structure of the muscles of the rotator cuff: an experimental study in sheep. J Bone Joint Surg Am 86-A:1973–1982.
15. Cofield RH, et al. 2001. Surgical repair of chronic rotator cuff tears. A prospective long-term study. J Bone Joint Surg Am 83-A:71–77.
16. Mellado JM, et al. 2005. Surgically repaired massive rotator cuff tears: MRI of tendon integrity, muscle fatty degeneration, and muscle atrophy correlated with intraoperative and clinical findings. AJR Am J Roentgenol 184:1456–1463.
17. Markov MS. 2007. Expanding use of pulsed electromagnetic field therapies. Electromagn Biol Med 26:257–274.
18. Mooney V. 1990. A randomized double-blind prospective study of the efficacy of pulsed electromagnetic fields for interbody lumbar fusions. Spine (Phila Pa 1976) 15:708–712.
19. Chalidis B, et al. 2011. Stimulation of bone formation and fracture healing with pulsed electromagnetic fields: biologic responses and clinical implications. Int J Immunopathol Pharmacol, 24:17–20.
20. Yan JL, et al. 2015. Pulsed electromagnetic fields promote osteoblast mineralization and maturation needing the existence of primary cilia. Mol Cell Endocrinol 404:132–140.
21. Barnaba S, et al. 2013. Effect of pulsed electromagnetic fields on human osteoblast cultures. Physiother Res Int 18:109–114.
22. Jansen JH, et al. 2010. Stimulation of osteogenic differentiation in human osteoprogenitor cells by pulsed electromagnetic fields: an in vitro study. BMC Musculoskelet Disord 11:188.
23. Fu YC, et al. 2014. A novel single pulsed electromagnetic field stimulates osteogenesis of bone marrow mesenchymal stem cells and bone repair. PLoS ONE 9:e91581.
24. Tsai MT, et al. 2009. Modulation of osteogenesis in human mesenchymal stem cells by specific pulsed electromagnetic field stimulation. J Orthop Res 27:1169–1174.
25. Boopalan PR, et al. 2011. Pulsed electromagnetic field therapy results in healing of full thickness articular cartilage defect. Int Orthop 35:143–148.
26. Choi MC, Cheung KK, Li X., et al. 2015. Pulsed electromagnetic field (PEMF) promotes collagen fibre deposition associated with increased myofibroblast population in the early healing phase of diabetic wound. Arch Dermatol Res. 307:925–935.
27. Osti L, Buono AD, Maffulli N. 2015. Pulsed electromagnetic fields after rotator cuff repair: a randomized, controlled study. Orthopedics 38:e223–e228.
28. Busch F, et al. 2012. Resveratrol modulates interleukin-1beta-induced phosphatidylinositol 3-kinase and nuclear factor kappaB signaling pathways in human tenocytes. J Biol Chem 287:38050–38063.
29. Bernardi H, et al. 2011. Wnt4 activates the canonical beta-catenin pathway and regulates negatively myostatin: functional implication in myogenesis. Am J Physiol Cell Physiol 300: C1122– C1138.
30. Sharma P, Maffulli N. 2005. Tendon injury and tendinopathy: healing and repair. J Bone Joint Surg Am 87:187–202.

31. Greenough CG. 1996. The effect of pulsed electromagnetic fields on flexor tendon healing in the rabbit. J Hand Surg Br 21:808–812.
32. Robotti E, et al. 1999. The effect of pulsed electromagnetic fields on flexor tendon healing in chickens. J Hand Surg Br 24:56–58.
33. Lee EW, et al. 1997. Pulsed magnetic and electromagnetic fields in experimental achilles tendonitis in the rat: a prospective randomized study. Arch Phys Med Rehabil 78: 399–404.
34. Lin Y, et al. 1992. Effects of pulsing electromagnetic fields on the ligament healing in rabbits. J Vet Med Sci 54: 1017–1022.
35. Denaro V, et al. 2011. Effect of pulsed electromagnetic fields on human tenocyte cultures from supraspinatus and quadriceps tendons. Am J Phys Med Rehabil 90:119–127.
36. Yablonka-Reuveni Z. 1995. Development and postnatal regulation of adult myoblasts. Microsc Res Tech 30:366–380.
37. Campion DR. 1984. The muscle satellite cell: a review. Int Rev Cytol 87:225–251.
38. White TP, Esser KA. 1989. Satellite cell and growth factor involvement in skelmuscle growth. Med Sci Sports Exerc 21: S158–S163.
39. Abmayr SM, Pavlath GK. 2012. Myoblast fusion: lessons from flies and mice. Development 139:641–656.
40. Leon-Salas WD, et al. 2013. A dual mode pulsed electromagnetic cell stimulator produces acceleration of myogenic differentiation. Recent Pat Biotechnol 7:71–81.
41. Gumucio JP, et al. 2012. Rotator cuff tear reduces muscle fiber specific force production and induces macrophage accumulation and autophagy. J Orthop Res 30: 1963–1970.
42. Mendias CL, et al. 2015. Reduced muscle fiber force production and disrupted myofibril architecture in patients with chronic rotator cuff tears. J Shoulder Elbow Surg 24:111–119.
43. Oak NR, et al. 2014. Inhibition of 5-LOX, COX-1, and COX-2 increases tendon healing and reduces muscle fibrosis and lipid accumulation after rotator cuff repair. Am J Sports Med 42:2860–2868.
44. Yao L, et al. 2011. Non-immortalized human tenocyte cultures as a vehicle for understanding cellular aspects to tendinopathy. Transl Med UniSa 1:173–194.
45. Yao L, et al. 2006. Phenotypic drift in human tenocyte culture. Tissue Eng 12:1843–1849.