sam® Professional *for Acute and Chronic Musculoskeletal Injury*

Appendix C: Technology and Innovation

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Biophysical, Cellular and Tissue Biomechanical Effects on Tissue

A significant body of research has focused on the biomechanical effects of daily-applied, low-intensity therapeutic ultrasound on soft tissue recovery. A review of peer-reviewed articles is provided below which examined the biophysical, cellular and tissue biomechanical effects of ultrasound on improving natural healing of soft tissue injuries to tendons, ligaments and muscles. The peer-reviewed literature shows that ultrasound facilitates tendon healing, with increased tensile strength and improved collagen alignment. For ligament and skeletal muscle injuries, ultrasound improves tissue biomechanics (ultimate load, stiffness, energy absorption), and increases cell proliferation during myoregeneration. Scientific evidence supports the use of ultrasound to treat soft tissue injuries, and this research is translated effectively with the *sam® Professional System*.

***sam® Professional* Provides Clinically Effective Ultrasound for Tendinopathies:** Low-intensity ultrasound intervention provides a beneficial effect on tendon strength and collagen synthesis following injury (Table 1). Placebo controlled scientific investigations demonstrate that the biomechanical strength of tendons treated with ultrasound is significantly greater than controls from 5 to 42 days post-injury ($p<0.05$; (10-12, 22, 36, 37); Figure 6). Tensile strength and tendon extensibility in ultrasound-treated tendons are also higher than in control tendons (10, 36-40). Collagen synthesis, measured by conversion of radiolabeled proline to hydroxyproline, increased substantially with ultrasound from day 3 to day 5 post-injury and continued to show benefits through day 21 compared to controls ($p<0.05$; (11, 12); Figure 7). Collagen type I and III expression is greater following ultrasound treatment (41), and higher birefringence (i.e., coherence of collagen alignment) is also observed in treated tendons compared to controls (42, 43). Additionally, an examination of treatment time and duration found that introducing ultrasound intervention in the earlier stages of healing increases tensile strength and matrix synthesis (22, 44). In human clinical studies examining epicondylitis and patellar tendinopathy, tendon pain decreased significantly over the course of 12 weeks when treated with ultrasound (5, 8).

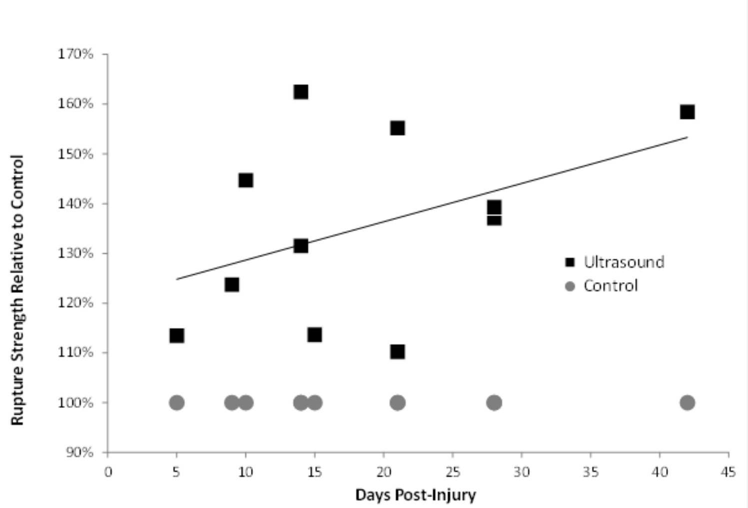

Figure 6: Low-intensity ultrasound significantly improves biomechanical heal of injured tendon over control (p<0.05).

Ligament Healing also Benefits from the Application of sam® Professional: Table 2 summarizes biophysical effects data for low-intensity ultrasound therapy. Ultrasound treated ligaments exhibited superior mechanical properties including ultimate load, stiffness, and energy absorption (45-47). Ultrasound-treated ligaments from one study were 34.2% stronger, 27.0% stiffer, and could absorb 54.4% more energy compared to sham-treated ligaments after 2 weeks of treatment (47). Another study demonstrated that after six weeks of ultrasound treatment, ligaments were 39.5% stronger, 24.5% stiffer, could absorb 69.1% more energy, and were 10.6% larger than sham-treated ligaments (45). Collagen fibril diameter was also larger in a group treated with ultrasound compared to controls (46), and there was a greater relative proportion of type I collagen in ultrasound-treated ligaments compared to controls at both 3 and 6 weeks (45).

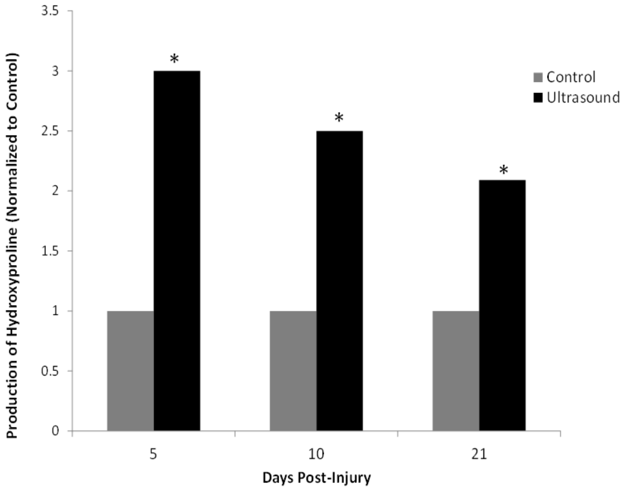

Figure 7: Low-intensity ultrasound significantly increases precursors of collagen production 2-3x in injured tendon tissue to accelerate healing ($p<0.05$).

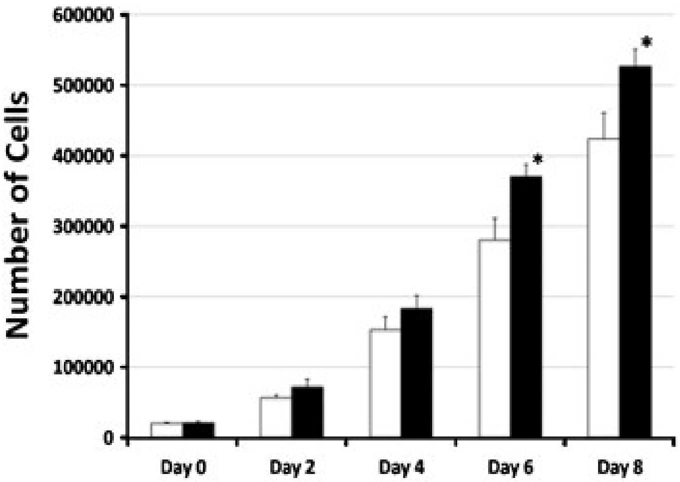

Figure 8: Low-intensity ultrasound significantly increases cellular proliferation in repairing muscle tissue ($p<0.05$).

Muscle Healing Effects from the Application of sam® Professional: Ultrasound intervention also increases cellular proliferation, and both myogenin and actin protein expression in skeletal muscle following a contusion injury (Table 3). Low-intensity ultrasound nearly doubles satellite cell proliferation compared to controls in injured gastrocnemius muscle (13). A higher proliferation rate and cell number at days 6 and 8 were observed following the application of therapy (Figure 8; (48)). Cells treated with ultrasound demonstrate a 40% increase in myogenin expression and a 47% increase in actin expression compared to controls (48). Cyclooxygenase-2 (COX-2) expression is also reduced in injured muscle tissue (both *in vivo* and *in vitro*) treated with ultrasound compared to controls (49, 50). Two human studies examining pain and trigger point depths demonstrated that ultrasound significantly reduces pain (6) and relaxes muscles (4).

Conclusion of Evidence Supporting the use of sam® Professional: The use of *sam® Professional* in the treatment of tendon, ligament and muscle healing is supported by the literature. The vast majority of biophysical, cellular and tissue biomechanical scientific data is based off of clinically relevant animal models where histological and biomechanical studies may be conducted in a controlled approach. The data summarized above supports the efficacy of sam® *Professional* intervention in the treatment of musculoskeletal injuries.

Table 1. Summary of Ultrasound in Tendon Healing

Author	Study Design	Results
Demir et al. 2004	84 male rats in 6 groups: 14 US, 14 sham-US, 14 laser, 14 sham-laser, 14 US + laser, 14 sham-US + sham-laser. Treated left tendon and right tendon served as control. *Outcome Measures:* Biochemical and Biomechanical	Hydroxyproline levels were significantly increased in the treatment groups compared to controls at 10 and 21 d. Tendon breaking strength of treatment groups were significantly increased compared to control groups.
Enwekema et al. 1990	24 rabbits in 2 groups: 10 US, 14 sham-US. Treated left tendon and right tendon served as control. *Outcome Measures:* Biomechanical (tensile strength, stress, energy absorption)	Tensile strength of US-treated tendons significantly greater than control tendons (49.3 vs. 29.9 N). Tensile stress of treated tendons significantly greater than control tendons (150.7 vs. 101.8 N cm^{-2}). Energy absorption capacity significantly greater in treated tendons than control tendons (189.8 vs. 55.8 mJ).
Fu et al. 2008	60 rats in 10 groups of 6: sham-US 1-14 d w/ harvest at 14, 28, or 42 d; US 1-14 d w/ harvest at 14, 28, or 42 d; US 14-28 d w/ harvest at 42 d; US 28-42 d w/ harvest at 42 d; US 1-28 d w/ harvest at 42 d, US 14-42 d w/ harvest at 42 d. *Outcome Measures:* Histology (hematoxylin and eosin staining), biomechanical	Daily US for 2 wks starting 1 d post-injury led to significant improvement in tensile strength at 42 d over sham-US (36.6 vs. 23.1 MPa). Tensile strength was also significantly improved in tendons treated during the first 2 wks post-injury compared to starting treatment at day 14 (31.8 MPa) or 28 (25.4 MPa). Cellularity and collagen stainability decreased progressively in US groups at later stages of healing (28 and 42 d post-injury). Significantly enhanced restoration of collagen birefringence in US-treated tendons vs. controls.
Fu et al. 2010	78 rats in 15 groups: Treated with US or sham-US on 4, 14, or 28 d post-injury. Tendon samples harvested at 4 h and 24 h post-sonication. *Outcome Measures:* mRNA expression of COL1A1, COL3A1, decorin, biglycan, TGFβ1	US treatment 4 d and 14 d post-injury significantly increased COL1A1 and COL3A1 mRNA expression compared to controls, but not at 28 d. Biglycan mRNA significantly decreased following US at 28 d post-injury, but no significant difference at 4 d or 14 d. Change in decorin mRNA expression not significant at 4 d, but significantly increased at 14 d and decreased at 28 d. Significantly increased TGFβ1 mRNA at 4 d but not 14 d or 28 d. At 28 d, alignment of collagen fibers and tendon cells became evident. US enhanced collagen synthesis during the granulation phase but not during the remodeling phase of tendon healing.
Jackson et al. 1991	55 rats in 6 groups: 16 uninjured controls, 6 injured controls, 27 injured rats received US for 2, 5, 9, 15, or 21 d, and 6 injured rat received US for 3 or 5 d. *Outcome Measures:* Biomechanical (breaking strength), collagen synthesis (rate of incorporation of labeled proline into hydroxyproline)	Breaking strength of treated tendons was significantly greater than untreated tendons 5, 9, 15, and 21 d post injury. Collagen synthesis was increased in treated tendons compared to untreated tendons 5 d post-injury: 2,000-24,000 counts per min/mmol hydroxyproline in treated tendons vs. 2,500 - 8,000 in untreated tendons from 3 to 5 d post-injury.
Jeremias et al. 2011	28 rats: 14 sham-US, 14 US. *Outcome Measures:* Biomechanical (cross-sectional area, ultimate load, tensile strength, energy absorption), expressed in comparison to non-injured contralateral tendon	Significantly greater ultimate load in US-treated tendons vs. controls (33.3% vs. -3.5%). Significantly increased tensile strength in US group vs. control group (47.7% vs. -28.1%).
Koeke et al. 2005	40 male rats in 5 groups: 8 uninjured controls, 8 sham-US, 8 sham-US + hydrocortisone cream, 8 US, 8 US + hydrocortisone cream. *Outcome Measures:* Birefringence	Concentration, state of aggregation, orientation, and deposition of collagen fibers were best in tendons treated with US + hydrocortisone, followed by US alone. Both showed significantly enhanced birefringence compared to controls and hydrocortisone alone. Hydrocortisone alone did not show any differences compared to controls.
Kosaka et al. 2011	98 rats (1 limb US, 1 limb sham US) were sacrificed at 0, 2, 5, 7, 14, 28 days. *Outcome Measures:* Electron microscopy examination, COX-2, EP4, collagen type I and III, and TGFβ1 expression	Tendon repair was accelerated in the US group judged by electron microscopy. Both COX-2 and EP4 were over-expressed in the US-treated group in the inflammatory period, and TGFβ1 expression was markedly induced in US treated tendons followed by collagen I and III expression in the repair and reconstitution process.
Larsen et al. 2005	72 rabbits in 8 groups of 9: treated at intensities of 0 (control), 0.05, 0.1, 0.2, 0.5, 0.75, 1.0, or 2.0 W/cm^2. *Outcome Measures:* Biomechanical (rupture strain, rupture load, stiffness, collagen content)	Extensibility of healing tendons was significantly greater after sonication with US. A gradual decline in stiffness and collagen content was observed with increasing US intensity. No significant effect on load at rupture was observed between US and control groups.

Ng et al. 2003	30 rats in 3 groups: 9 sham-US, 10 US at 1.0 W/cm^2, 11 US at 2.0 W/cm^2. *Outcome Measures:* Biomechanical (load-relaxation, stiffness, strength), Achilles function index (AFI)	Ultimate tensile strength of US-treated tendon was significantly greater than controls. Load-relaxation and stiffness were not significantly different among groups. There were no significant differences in AFI between groups, but there was a trending drop for all groups at 3 d followed by gradual improvement.
Ng & Fung 2007	20 rats in 4 groups of 5: treated at intensities of 0 (control), 0.5, 1.2, or 2.0 W/cm^2. *Outcome Measures:* Collagen fibril size	Mean collagen fibril size of all treatment groups was significantly higher than the control group. The group treated with 0.5 W/cm^2 had the largest mean fibril diameter (205.3 nm) and the control group had the smallest (69.7 nm). There was no significant difference between fibril sizes among US treatment groups.
Warden et al. 2008	27 patients in 2 groups: 13 US, 14 sham-US. All performed eccentric exercises daily. *Outcome Measures:* Pain during usual and most aggravating activity (VAS), knee function (Victorian Institute of Sports Assessment; VISA), patient's perceived response to treatment (5-point scale)	The two groups had significantly decreased usual (Δ -1.7) and worst (Δ -2.5) knee pain over 12 weeks, as well as improved knee function on the VISA scale (Δ 12.3). 85% of US-treated patients and 64% of sham-treated patients felt their injury had improved.
Wood et al. 2010	50 rats in 5 groups: 10 control, 10 US alone, 10 low-level laser treatment (LLLT), 10 US then LLLT, 10 LLLT then US. *Outcome Measures:* Birefringence	Significantly better organization of collagen fibers in US alone group versus control. Type I collagen higher in US alone, LLLT alone, and LLT then US compared to controls.
Yueng et al. 2006	48 rats in 4 groups: 12 sham-US for 2 wks, 12 sham-US for 4 wks, 12 US for 2 wks, 12 US for 4 wks. *Outcome Measures:* Biomechanical (ultimate tensile strength, stiffness, load relaxation), histological (collagen growth evaluated using light microscopy)	Tensile strength and stiffness of the repaired tendon were higher in treatment groups than controls at 2 and 4 wks. There were no differences in load relaxation. At 2 wks, collagen was more regular, denser, and better aligned in US group compared to controls. At 4 wks, total area of collagen matrix was slightly higher in the US group than the control group.

Table 2. Summary of Ultrasound in Ligament Healing

Author	Study Design	Mechanical and Histology Results
Sparrow et al. 2005	21 rabbits in 2 groups: 10 sacrificed at 3 wks, 11 sacrificed at 6 wks; all animals had contralateral ligament serve as control. *Outcome Measures:* Biomechanical (ultimate load, ultimate displacement, energy absorption), biochemistry (collagen concentration, proportions)	US-treated ligaments were significantly larger at 6 wks. Ultimate load, ultimate displacement, and energy absorption were significantly higher than control ligaments at 6 wks. The relative proportion of type I collagen was significantly higher in US- vs. sham-treated ligaments at 3 and 6 wks.
Takakura et al. 2002	13 rats in 2 groups: 8 sacrificed at 12 d, 5 sacrificed at 21 d; all animals had contralateral knee serve as control. *Outcome Measures:* Biomechanical (ultimate load, stiffness, energy absorption), collagen fibril diameter (electron microscope)	US-treated sides showed significantly enhanced ultimate load, stiffness, and energy absorption, compared to control sides at 12 d, but not at 21 d. Mean diameter of collagen fibril significantly larger for US side compared to control side.
Warden et al. 2006	60 rats in 2 groups treated with NSAID vehicle or inert control vehicle, with contralateral limb serving as control (total of 120 limbs). Further divided as: 30 sham-US + control vehicle (controls), 30 US + control vehicle (US alone), 30 sham-US + NSAID vehicle (NSAID alone), 30 US + NSAID vehicle (US + NSAID). Of the 60 rats, 28 sacrificed at 2 wks, 16 at 4 wks, 16 at 12 wks. *Outcome Measures:*	At 2 wks, ligaments treated with US were 34.2% stronger, 27% stiffer, and could absorb 54.4% more energy compared to ligaments treated with sham-US. Ligaments from NSAID group absorbed 33.3% less energy than inert vehicle group.

Biomechanical (ultimate load, stiffness, energy absorption)

Table 3. Summary of Ultrasound in Healing of Skeletal Muscle

Author	Study Design	Mechanical and Histology Results
Chan et al. 2010	*In vitro:* C2C12 cells exposed to US or sham-US. Cell growth was evaluated on 2, 4, 6, and 8 d. *Outcome Measures:* Cell number, western blot 40 mice in 5 groups: 8 sham-US, 8 US for 7 d, 8 US for 14 d, 9 US for 21 d, or 8 US for 28 d. *In vivo: Outcome Measures:* Muscle regeneration and muscle contractile properties	US therapy produced a significantly higher proliferative rate and cell number after 8 d. Significant increase in myogenin and actin proteins in cells treated with US for groups at 4, 6, and 8 d. Significant increases in myogenin (40%) and actin (47%) for cells treated with 8 doses of US compared to control. Regeneration of myofibers in US treated muscles at 21 and 28 d greater than control group.
Draper et al. 2010	26 patients in 2 groups: 13 US, 13 sham-US. *Outcome Measures:* Trigger point depths before and after US treatment	Trigger point depths significantly increased in the US group compared to controls over the two week period, suggesting greater muscle relaxation.
Karnes et al. 2002	33 rats in 5 groups: 7 uninjured control, 4 sham-US, 7 US for 3 d, 8 US for 5 d, 7 US for 7 d. *Outcome Measures:* Maximum isometric tetanic force (FIM; functional index of muscle injury)	FIM was significantly greater in US group versus sham-US group at 7 d but not 3 or 5 d. Uninjured group had significantly greater FIM than treated and untreated rats that sustained injury.
Lewis et al. 2013	30 patients in 2 groups: 20 US, 10 sham-US. *Outcome Measures:* Pain (VAS), global functioning (Global Rate of Change; GROC)	There was a 1.94 reduction in pain and a 1.58 improvement in global health scores in the US group, which significantly differed from the placebo group on the first 2 d.
Nagata et al, 2013	*In vitro:* C2C12 cells were cultured with or without TNFα or IL-1B. *Outcome Measures:* RNA isolation, PCR, western blot analysis 48 mice: treated for 1, 3, 5, or 7 days; all animals had contralateral ligament serve as control. *In vivo: Outcome Measures:* RNA isolation, PCR, western blot analysis, histopathology, immunoflurorescence	Compared to control, US caused significant down-regulation of COX-2 mRNA expression. US significantly up-regulated myogenin mRNA and up-regulated myogenin protein synthesis above the level in untreated control. Increase of COX-2 mRNA with US at 1 d but a decrease at 5 d. At 5 d, increase in myogenin mRNA and protein, increase in fast myosin protein, and increase of paired-box transcription factor 7 positive cells in US-treated muscles. At 5 d, number of inflammatory cells was significantly decreased in US-treated muscles compared to controls. At 7 d, the cross-sectional area of myofibers treated with US was significantly larger than controls.
Rantanen et al. 1999	56 rats over 14 groups: 12 rats treated w/ US 3 d post-injury and 4/12 sacrificed at 4, 7, 10 d; 12 rats treated w/ sham-US 3 d post-injury and 4/12 sacrificed at 4, 7, 10 d; 16 rats treated w/ US 6 h post-injury and 4/16 sacrificed at 1, 3, 6, 9 d; 16 rats treated w/ sham-US 6 h post-injury and 4/16 sacrificed at 1, 3, 6, 9 d. *Outcome Measures:* Immunohistochemical, morphometric, and scintigraphic analyses	Satellite cell proliferation was enhanced significantly (up to 96%) by US during the early stages of regeneration. The period of rapid fibroblast proliferation was extended from 3 to 4 d in the control group to 7 to 10 d in the US group.
Renno et al. 2011	35 rats in 4 groups: 5 uninjured control, 10 sham-US, 10 low-level laser therapy (LLLT), 10 US. *Outcome Measures:* Immunochemistry, histopathology	COX-2 expression higher in sham-US group than in those treated with either US or LLLT. Histopathological examination found less extensive myofibrillary degeneration in the US and LLLT groups compared to the sham-US group.

Appendix D: References

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