Application of Shortwave Ultrasound to Tendon-like Constructs Cultured from Bone Marrow Derived Mesenchymal Stem Cells *in vitro* Produces Tissue of Greater Strength

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DOI: 10.1111/evj.54_12612

Morrell, H.P., Mackenzie, A.M., Brizuela, C.B. and Canty-Laird, E.G. 2016. Application of Shortwave Ultrasound to Tendon-like Constructs Cultured from Bone Marrow Derived Mesenchymal Stem Cells *in vitro* Produces Tissue of Greater Strength. *Equine Veterinary Journal*, 48(S50), pp.27-28.

Application of Shortwave Ultrasound to Tendon-like Constructs Cultured from Bone Marrow Derived Mesenchymal Stem Cells *in vitro* Produces Tissue of Greater Strength

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Journal:	*Equine Veterinary Journal - Abstracts*
Manuscript ID	Draft
Wiley - Manuscript type:	BEVA Congress 2016
Discipline:	Cell biology, Physiotherapy
Body System/Disorder:	Musculoskeletal: tendons and ligaments < Musculoskeletal
Abstract:	**Reasons**. There is a growing body of published research seeking to improve equine tendon healing processes and outcomes. **Objectives**. To determine whether there were biomechanical benefits in applying therapeutic shortwave ultrasound alongside bone marrow derived stem cell infusion, through an *in vitro* 3-D tendon construct model. **Study design.** Controlled *in vitro* study. Methods. Equine BM-MSCs were cultured within optimum conditions for differentiation and proliferation into 3-D constructs with application of different levels of therapeutic ultrasound (control, 0.1W/cm^2, 0.4W/cm^2, 0.8W/cm^2). Histological examination of the constructs was performed, together with biomechanical testing for breaking strain. Results were analysed using ANOVA and polynomial contrasts. **Results**. Constructs treated with 0.1W/cm^2 and 0.4W/cm2 had formed at a more rapid rate than control or 0.8W/cm^2 samples ($p<0.05$). H&E stained histological sections demonstrated a four-fold increase in cell number *cf*. control for 0.1W/cm^2 treatment group ($p=0.019$). Construct diameter was significantly greater for constructs receiving ultrasound treatment ($p<0.001$). Significant quadratic relationships were found between ultrasound dose level and breaking strain ($P=0.028$), with the optimum level of ultrasound between 0.1 and 0.4W/cm^2. Constructs exposed to ultrasound at powers of 0.4W/cm^2 and 0.8W/cm^2 lacked structural integrity. More mature cell formation was noted in 0.1w/cm^2 and 0.4W/cm^2 groups, with cells migrating to the edges of the construct adopting a spindle shape aligned to the unipolar forces applied, with evidence of crimping. **Conclusions**. Application of low level therapeutic ultrasound to BM-MSCs *in vitro* was not detrimental to cell proliferation or differentiation. Culture was accelerated, producing constructs of greater cross-sectional area and evidence of greater levels of cell maturity, resulting in

	significantly higher ultimate breaking strain that seen in control groups or those treated with high power.

Clinical Research Abstracts of the British Equine Veterinary Association Congress 2016

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Reasons for performing study. Equine tendon injury is a major loss to the industry, consequently there is a growing body of published research seeking to improve tendon healing processes and outcomes. **Objectives.** To determine whether there were biomechanical benefits in applying therapeutic shortwave ultrasound alongside bone marrow derived stem cell infusion, through an *in vitro* 3-D tendon construct model. **Study design.** Controlled *in vitro* study. **Methods.** Equine BM-MSCs were cultured for a period of 24 days within optimum conditions for differentiation and proliferation into 3-D constructs with the application of different levels of therapeutic ultrasound (control, 0.1W/cm^2, 0.4W/cm^2, 0.8W/cm^2) on alternate days. Histological examination of the constructs was performed at day 24, together with biomechanical testing for breaking strain. Results were analysed using ANOVA and polynomial contrasts to assess breaking strain dose response. **Results.** By day 16, constructs treated with 0.1W/cm^2 and 0.4W/cm^2 had formed at a more rapid rate than control or 0.8W/cm^2 samples ($p<0.05$). H&E stained histological sections demonstrated a significant four-fold increase in cell number *cf.* control for 0.1W/cm2 treatment group (p=0.019). Construct diameter was significantly greater for all constructs receiving ultrasound treatment ($p<0.001$). Significant quadratic relationships were found between ultrasound dose level and breaking strain (P=0.028), with the optimum level of ultrasound between 0.1 and 0.4W/cm^2. Histological examination revealed constructs exposed to ultrasound at powers of 0.4W/cm2 and 0.8W/cm2 lacked structural integrity, with disruption to cell alignment and the biological scaffold. More mature cell formation was noted in 0.1w/cm2 and 0.4W/cm2 groups, with cells migrating to the edges of the construct and adopting a spindle shape aligned to the unipolar forces applied, with some evidence of crimping. **Conclusions.** Application of low levels of therapeutic ultrasound to BM-MSCs *in vitro* was not detrimental to cell proliferation or differentiation. Resulting culture was accelerated, producing constructs of greater cross-sectional area and evidence of greater levels of cell maturity, resulting in significantly higher ultimate breaking strain that seen in control groups or those treated with high power. **Ethical animal research:** BM-MSCs were collected under Home Office Licence **Source of funding:** Horserace Betting Levy Board, National Association of Veterinary Physiotherapists **Competing interests:** None declared.