Non-invasive Electroarthrography Correlates to Direct Measurements of Cartilage Streaming Potentials in Weight Bearing Regions of Equine Metacarpophalangeal (Fetlock) Joints

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Introduction

• Degenerative joint diseases, like osteoarthritis, are characterized by progressive cartilage degeneration, which can lead to pain and loss of mobility[1-2].

• Low-grade cartilage deterioration occurs early in disease progression and may be treatable[2].

• However, current clinical assessment methodologies, including physical exam, synovial fluid analysis and imaging, may not be sensitive enough to detect early degenerative changes[3-6].

•Electroarthrography (EAG) is a new technology capable of measuring streaming potentials produced by cartilage during compression through electrodes applied to skin surrounding an articular joint[7-8].

• Streaming potentials arise from interactions among constituents of the cartilage extracellular matrix during load bearing and provide a sensitive measure of cartilage degeneration[9-11].

• Consequently, EAG may provide a sensitive, non-invasive method for detecting low-grade cartilage degeneration. The relationship between EAG and direct measurements of cartilage streaming potentials was explored here in equine fetlock joints.

Hypotheses

• EAG, which assesses streaming potentials externally, correlates to direct measurements of streaming potentials in weight bearing cartilage.

• EAG can distinguish between fetlocks obtained from a horse with a clinical history of joint disease and those of young, normal horses.

Methods

1. Equine Fetlock Joint Explants (n=6)

•13 year old horse (n=2): History of forelimb joint disease.
•3 year old horses (n=4): Racehorses with no reported musculoskeletal issues.

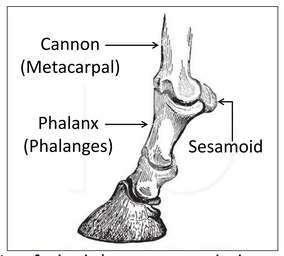

Fig. 1: Equine fetlock (metacarpophalangeal) joint[12]

2. EAG during Simulated Standing & Walking

•Each fetlock mounted in a mechanical tester (Instron 8000) and aligned with the axes of a six degree of freedom load cell.

•10 gold-plated electrodes (10 mm diameter) attached to skin at 6 sites around the fetlock and at negative reference and ground sites (**Fig. 2**).

•Sites identified by palpation and prepared by removing hair, lightly abrading skin and cleaning with alcohol.

•Standing and walking loads[13] were approximated with displacements of 15 mm and 25 mm, respectively.

•Load sequences consisted of 10 cycles. During each cycle, displacement was applied at 5 mm/s, held for 5 s, and unloaded at 5 mm/s.

•EAG signals were acquired at 600 Hz with a wireless data acquisition system (Clevemed Bioradio 150).

3. Direct Assessment of Cartilage Streaming Potentials

•Fetlocks disarticulated and cartilage appearance assessed with India ink.

•Direct measurements of cartilage streaming potentials made with the Arthro-BST device (**Fig. 3**) at 138 ± 6 (n=6) and 101 ± 7 (n=6) sites on the cannon and phalanx, respectively.

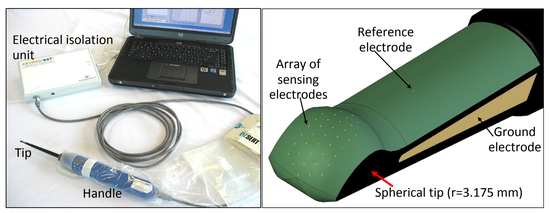

Fig. 3: The Arthro-BST is an arthroscopic device that directly measures cartilage streaming potentials by compressing cartilage with a spherical indenter containing an array of 37 gold microelectrodes (5 microelectrodes/mm^2).

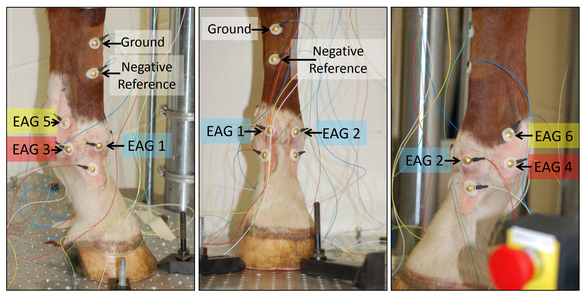

Fig. 2: Left fetlock prepared for EAG. Anterior electrodes placed at medial (EAG1) & lateral (EAG2) cannon/phalanx interface. Medio-lateral electrodes placed at medial (EAG3) & lateral (EAG4) cannon/phalanx interface. Sesamoid electrodes placed at medial (EAG5) & lateral (EAG6) cannon/sesamoid interface. Negative reference & ground electrodes attached to the cannon away from the articulation.

4. Data & Statistical Analyses

•**EAG coefficients (μV/kg):** Determined by fitting EAG signals to measured axial loads for each electrode. EAG coefficients from load cycles 6-10 were averaged.
•**Quantitative Parameter (QP):** Calculated by the Arthro-BST and corresponds to the number of microelectrodes in contact with cartilage when the sum of streaming potentials reaches 100 mV.
•**Weight Bearing Areas:** QP grouped according to weight bearing areas identified from reported load distribution patterns[13-15] (**Fig. 5**).
•**Statistical Analyses (Statistica 8.0):** Pearson's correlations calculated between EAG coefficients and QP in weight bearing areas. Normal and degraded fetlocks compared with a one-way ANOVA and Tukey's HSD.

What are Streaming Potentials?

•During cartilage compression, positive mobile ions in the interstitial fluid are displaced relative to the fixed negatively-charged proteoglycan molecules, which are immobilized in the collagen network (Fig. 4A).

•In osteoarthritic cartilage (Fig. 4B), the collagen network is degraded and there is a loss of proteoglycans, leading to abnormal streaming potentials.

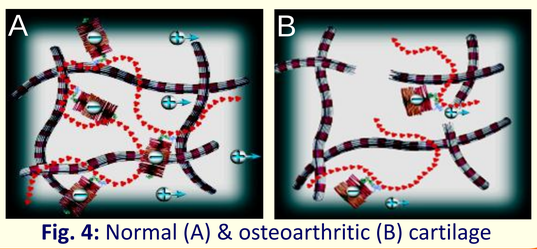

Fig. 4: Normal (A) & osteoarthritic (B) cartilage

Results & Discussion

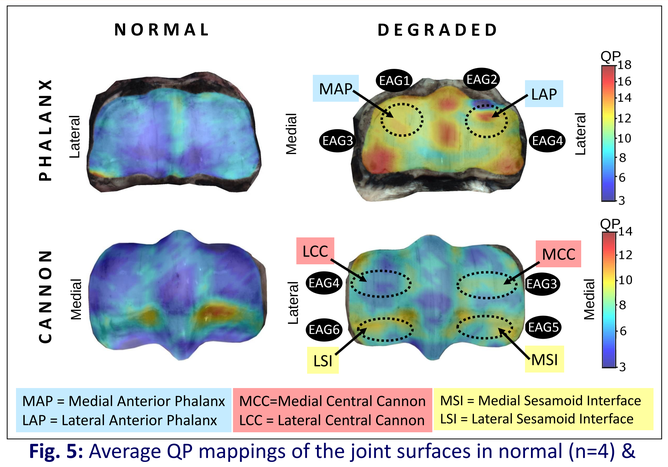

Fig. 5: Average QP mappings of the joint surfaces in normal (n=4) & degraded (n=2) fetlocks. QP is inversely proportional to cartilage stiffness & higher QP represents softer or degraded cartilage. Weight bearing areas & approximate locations of electrodes placed on skin external to the articulation are identified.

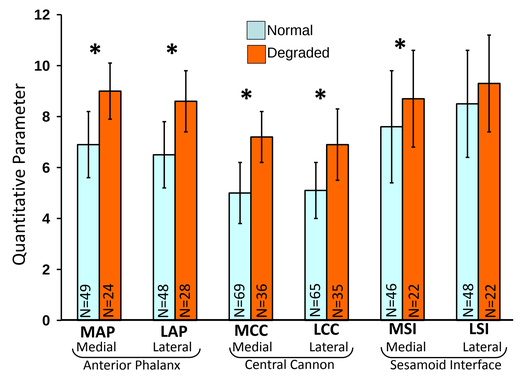

Fig. 6: QP (AVG ± SD) obtained by directly indenting cartilage in weight bearing areas defined in Fig. 5. (N) indicates total number of measurements per area in normal (n=4) & degraded (n=2) fetlocks. (*) indicates p<0.05. QP is inversely proportional to cartilage stiffness meaning higher values represent degradation.

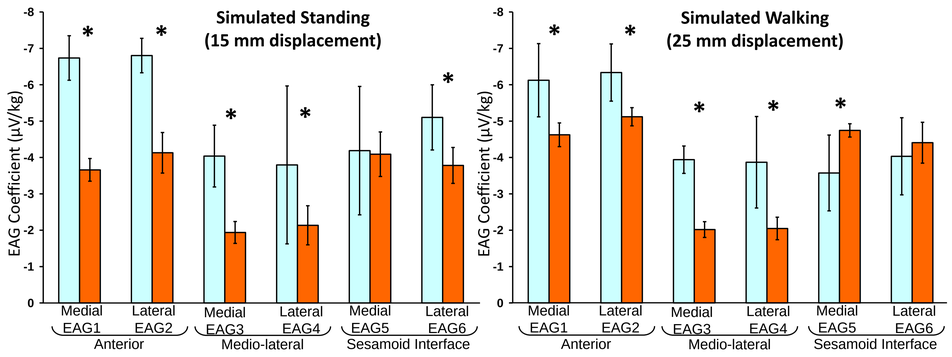

Fig. 7: EAG coefficients (AVG ± SD) for normal (n=4) & degraded (n=2) fetlocks calculated from the last 5 cycles of each load sequence for a total of 20 cycles for normal & 10 cycles for degraded fetlocks. (*) indicates p<0.05.

Quantitative Parameter: Direct Streaming Potentials

EAG Coefficients: External Streaming Potentials	Simulated Standing: Anterior Phalanx (MAP, LAP)	Simulated Standing: Central Cannon (MCC, LCC)	Simulated Standing: Sesamoid Interface (MSI, LSI)	Simulated Walking: Anterior Phalanx (MAP, LAP)	Simulated Walking: Central Cannon (MCC, LCC)	Simulated Walking: Sesamoid Interface (MSI, LSI)
Anterior Electrodes (EAG1, EAG2)	**0.81 (p=.002)**	**0.90 (p<.001)**	0.28 (p=.380)	0.53 (p=.073)	**0.63 (p=.027)**	-0.05 (p=.887)
Medio-lateral Electrodes (EAG3, EAG4)	0.50 (p=.096)	**0.64 (p=.025)**	**0.58 (p=.049)**	**0.63 (p=.029)**	**0.78 (p=.002)**	0.48 (p=.110)
Sesamoid Electrodes (EAG5, EAG6)	0.20 (p=.533)	0.26 (p=.420)	-0.16 (p=.612)	-0.48 (p=.118)	-0.43 (p=.168)	**-0.84 (p=.001)**

Fig. 8: Pearson's correlations (r) between indirect (EAG) & direct (QP) measurements of cartilage streaming potentials. QP were averaged over weight bearing areas defined in Fig. 5.

• Degraded fetlocks exhibited lower (p<0.05) EAG coefficients at anterior (EAG1, EAG2) and medio-lateral (EAG3, EAG4) electrodes (Fig. 7), as well as higher QP (p<0.05) measured directly in corresponding weight bearing areas of the anterior phalanx and central cannon (Fig. 5 & Fig. 6).

• EAG and QP changes were less consistent at the cannon/sesamoid interface. EAG coefficients in degraded fetlocks were reduced at EAG6 (p<0.001) but not EAG5 during standing, and increased at EAG5 (p=0.002) but not EAG6 during walking (Fig. 7). Similarly, QP increased (p<0.05), indicating softer cartilage, in the corresponding medial but not lateral weight bearing cartilage (Fig. 5 & Fig. 6).

• Streaming potentials are directly proportional to cartilage compressive stiffness and correlations between EAG and QP in weight bearing cartilage follow known load distribution patterns in the fetlock[13-15] (Table 1).

• Specifically, correlations at anterior (EAG1, EAG2) and medio-lateral electrodes (EAG3, EAG4) correlated to QP in the anterior phalanx and central cannon (Table 1, Fig. 5), which corresponds to Brama *et al.*[13] who used pressure film to demonstrate that the highest compressive stresses occur on the anterior phalanx.

• A strong correlation was also observed at the sesamoid interface during walking (Table 1) but not standing, which may result from increased involvement of these joint surfaces in load bearing at higher flexion angles[15].

• Streaming potentials, assessed indirectly by EAG and directly with the Arthro-BST, distinguished differences in cartilage quality consistent with the clinical history of these horses, although similar features of mild cartilage degradation were observed with India ink on both degraded and normal fetlocks.

Conclusions & Significance

• Externally-measured EAG correlated to direct measurements of cartilage streaming potentials in weight bearing cartilage of equine fetlock joints.

• These data support the development of EAG into a non-invasive, clinical methodology for cartilage health that could contribute to the early detection and treatment of osteoarthritis and other degenerative joint diseases.

Acknowledgements: Natural Sciences and Engineering Research Council of Canada

References: **[1]** Mandelbaum & Waddell Orthop 2005; 28(2 Suppl):207. **[2]** Lotz & Krauss Arthritis Res Ther 2010; 12(3):211. **[3]** Eckstein *et al.* Curr Opin Rheumatol 2007; 19(5):435. **[4]** Kijowski *et al.* Radiol 2006; 239(3):818. **[5]** Buckwalter Clin Sports Med 2006; 25(4):899. **[6]** Oliviero *et al.* Joint Bone Spine 2013; 80(3):287. **[7]** Savard & Buschmann US Patent 2011/0034797 A1. **[8]** Préville *et al.* Osteoarthr Cartil 2013; 21:1731. **[9]** Légaré *et al.* J Orthop Res 2002; 20(4):819. **[10]** Bonassar *et al.* Acta Orthop Scand Suppl 1995; 266:38. **[11]** Changoor *et al.* J Biomech Eng 2011; 133(6):061005. **[12]** Picsburg Illustrationsof.com/1130593. **[13]** Brama *et al.* Equine Vet J 2001; 33(1):26. **[14]** Neundorf *et al.* Am J Vet Res 2010; 71(11):1284. **[15]** Santschi Vet Clin Equine 2008; 24:117.