ESTIMATION AND SENSITIVITY TO CARTILAGE DEGRADATION OF ELECTROMECHANICAL COUPLING COEFFICIENTS DERIVED USING SPATIALLY RESOLVED STREAMING POTENTIALS

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Introduction: Streaming potentials are generated in charged connective tissues due to load-induced motion of mobile-ion containing interstitial fluid relative to fixed macromolecular charge of the extracellular matrix (ECM). Since streaming potentials reflect both the amount of charged proteoglycan and the extent to which it is immobilized by the collagen network, they are sensitive non-destructive indicators of cartilage health versus disease [1]. In a linear, homogeneous, biphasic tissue undergoing infinitesimal strains, the local streaming potential, ϕ, is related to local interstitial fluid pressure, p, by the electromechanical coupling coefficient Ke via $\phi=Ke*p$. Ke is therefore an intrinsic tissue property representing proteoglycan content and immobilization within the collagen network. Ke has been measured previously using permeation [2] and confined compression [3,4]; both of these techniques require destructive excision of the tissue. In this study we show how Ke may be obtained non-destructively using spatially resolved streaming potentials combined with a simultaneous force measurement. The ability of this technique to monitor tissue degradation is demonstrated and certain advantages relative to single potential or single force measurements are shown.

Methods: Cartilage Explant Isolation: Cartilage disks (4mm dia.) were isolated from the humeral head of a 1-2 year old steer retaining a thin bone layer. All disks were frozen at -20°C for 2 months prior to testing. Apparatus: A linear array of 8 platinum wire electrodes of 50µm diameter separated by 300µm was incorporated into the non-conductive base of a micromechanical testing chamber and then mounted on a mechanical tester (Mach1™, Biosyntech) [5]. Electromechanical Testing: After thawing at room temperature (~1 hour) in the first testing medium (NaCl at 0.01M) disks were punched to 3 mm diameter. The disk articular surface was placed in contact with the electrode array oriented with one electrode in the bath at the periphery of the disk while the remaining 7 electrodes were on the disk diameter. The electrical potentials on the surface of the disk were measured relative to the electrode in the bath. Test Procedure: After contacting the bone layer with a post connected to the load cell, a 100µm static compression offset was applied and the chamber filled with the saline solution (~10ml). After equilibrium (~45 min), a trapezoidal ramp compression of 300µm amplitude at 10µm/s with a 1 second wait at the top followed by release at 10µm/s was applied. All disks were tested with this procedure in baths of 0.01M, 0.1M, 1M NaCl and a PBS solution (n=5). After the last test in PBS, time to reach equilibrium (~45 min) was allowed, and either PBS was changed for new PBS solution containing 1mg/ml of trypsin (n=3) or wasn't changed to serve as a control (n=2). Then a ramp/release test was performed every hour for 12 hours. Electromechanical Analysis: The analysis is based on an expression from biphasic theory [6] $\sigma'=-(1+\alpha)pI+\sigma^E$ (Eq.1) where σ' is the total stress tensor in the tissue, α is the ratio of the solid to fluid volume (assumed here to be 0.25), p is the fluid pressure, I is the identity tensor and σ^E is the elastic stress of the solid phase. We neglect the σ^E term in the transient response to large deformation and the load is obtained by integrating Eq.1 across the surface of the tissue, $F=\int_S -\sigma' ds=(1+\alpha)2\pi\int p(r)rdr$. Substituting $p(r)=\phi(r)/Ke$ we obtain $F=2\pi(1+\alpha)\int\phi(r)rdr/Ke$ and therefore $Ke=SPI/F$ where $SPI=2\pi(1+\alpha)\int\phi(r)rdr$ is calculated using the spatially resolved streaming potential map $\phi(r)$ (example in Fig 2). Thus the local relation with $Ke=\phi(r)/p(r)$ is transformed into the above global relation $Ke=SPI/F$ and is easily calculated from measured forces and potentials since at any particular instant the slope of SPI vs F renders Ke.

Results: Streaming potentials measured along the disk radius, with reference to the bath potential, revealed a form versus time similar to that of the load, for these high amplitude compression/release profiles (Fig 1). The potential is negative due to the negative fixed charge of the cartilage matrix and the potential amplitude decreases from the center of the disk to its periphery following the form of the expected fluid pressure profile (Fig 2). Plotting the potential integral versus force during the compression and release phases showed a decreasing Ke (slope) during compression but a constant Ke during release (Fig 3). The following results were obtained using the constant Ke in release. Ke was sensitive to ionic strength, decreasing substantially as salt concentration was increased (Fig 4) and agreed with previously published trends [3]. Ke was also a good indicator of trypsin-induced degradation decreasing threefold over 12 hours (Fig 5).

Discussion: The goal of this study was to develop a non-destructive measurement of cartilage integrity by estimating the electromechanical coupling coefficient using force and electrical potential sensors. Since Ke is the ratio of potential to force, it possesses several practical advantages relative to measuring either potential or force alone, in particular its insensitivity to cartilage thickness, and to the amplitude and speed of the imposed displacement. For example when using a clinical arthroscopic device to apply displacement to an articular surface, the need to apply a finely controlled displacement is reduced as is the need to know cartilage thickness in order to interpret the results. Of course these advantages are ideally provided when model assumptions are respected including linearity, homogeneity and material properties independent of strain and strain-rate. In this study we have shown the ability of the technique to sensitively and non-destructively follow enzymatic degradation thus demonstrating its potential to be applied clinically to diagnosis and assess treatments of cartilage pathology.

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Fig 1 Streaming Potentials and Load vs Time

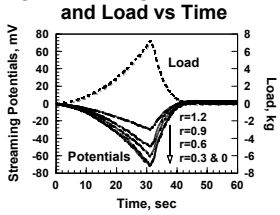


Fig 2 Streaming Potentials vs Radius at Peak Load

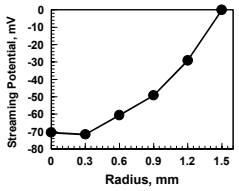


Fig 3 Streaming Potential Integral vs Force

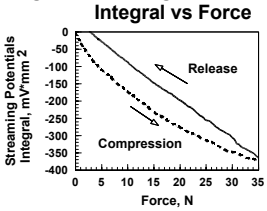


Fig 4 *Ke* vs NaCl Concentration

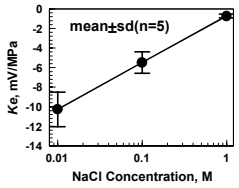
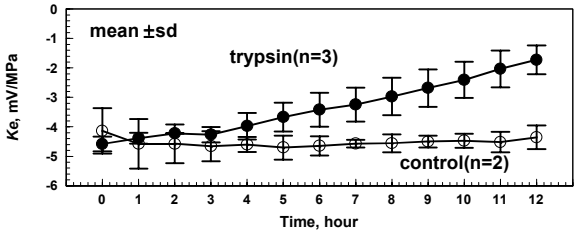


Fig 5 *Ke* vs Time



References: [1] Bonassar LJ et al., *Arch. Biochem. Biophys.* 333:359, 1996. [2] Maroudas A, *Biophys. J.* 8:575,1968. [3] Frank EH and Grodzinski AJ, *J. Biomech.* 20:615,1987. [4] Chen AC et al., *Ann. Biomed. Eng.* 25:269, 1997. [5] Garon et al., *J. Biomech.* Submitted ,1999. [6] Mow VC et al., *J.Biomech. Eng.* 102:73, 1980.